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VOL. 49 JULY 1, 1919 No. 2

STUDIES IN SECONDARY TRAUMATIC SHOCK

II. Shock due to Mechanical Limitation of Blood Flow

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INTRODUCTORY

The first phase of our study of the shock problem consisted in an examination of the mechanical changes in the circulation occurring in shock as induced by procedures directly involving the abdominal viscera, that is to say, in the type of shock most commonly investigated (1). Another method of inducing shock that has received some attention is the so-called mechanical shock of Janeway and Jackson (2). These investigators have studied the effect of temporary, partial occlusion of the inferior vena cava, and on the basis of their data have drawn certain conclusions with regard to the mechanism of the circulatory disturbance resulting therefrom, and with regard to its bearing on the problem of shock in general.

We have produced, and have studied by our methods, this type of shock. In the effort to interpret the results obtained, we have also included in our program a study of the mechanical changes in the circulation induced by partial, temporary obstruction of the aorta. Our experiments on the effects of temporary anemia induced in these two ways form the subject of the present paper.

SHOCK BY TEMPORARY PARTIAL OCCLUSION OF THE INFERIOR VENA CAVA

The method employed by Janeway and Jackson consisted in placing a thread around the cava in the thorax and then reducing the arterial pres-

sure to 30 to 40 mm. Hg. for 2 hours by means of graded tension exerted upon the vein through the thread. They found that after deocclusion, shock developed within 18 hours, the arterial pressure in the meanwhile sometimes recovering even the normal level.

The method of obstructing the vena cava finally adopted by us differed somewhat from that of Janeway and Jackson in that, instead of opening the chest and laying a ligature around the cava, a clamp was devised by which graded compression could be exerted upon the cava between the liver and the diaphragm through a small abdominal incision. Our method of following the tone of the peripheral arteries (1) gives the best results when shock develops within 5 or 6 hours. In many instances a period of occlusion lasting 2 hours sufficed to bring on shock within this limit. But not infrequently it became obvious, soon after deocclusion, that the failure of the circulation was not going to run its course within the optimum time limit of the method. In such instances, in order to hurry the process, the clamp was again applied to the cava for periods which were varied in duration to suit the needs of the case. In other instances, the onset of circulatory failure was hastened by holding the arterial pressure lower than 30 to 40 mm. Hg. It will be seen as the subject is developed that, in so far as the experiments overlap, they essentially confirm Janeway and Jackson. The methods employed in following the arterial, the systemic and portal venous pressures, and the peripheral resistance have been described in the preceding paper (1).

Experimental data

The results we have obtained have not been uniform enough to lend themselves to illustration by a single type experiment. Instead, it will be necessary to describe and discuss individually the results of several of the experiments before making an attempt to generalize.

Experiment 4 (*fig. 1*). By means of a thread passed around the cava through an opening in the thorax, the arterial pressure was held down at first to 30 mm. Hg., and later to 18 mm. Hg. During this period the inflow rate (femoral) at first was slowed somewhat (asphyxial constriction) but soon it began to increase and this increase continued steadily up to the reading made immediately after deocclusion (loss of constrictor tone). After deocclusion the arterial pressure rose momentarily as high as 55 mm. Hg., but otherwise was very low until the reading made at 3:17. Throughout this period the inflow rate remained high. Then, for a period of about 50 minutes, the arterial pressure remained above 50 mm. Hg., for a time reaching 64 mm. Hg. The inflow rate here steadily diminished and by

4:25 had practically reached the normal level (partial recovery of vasomotor tone). But now, coincidently with a rapid fall in arterial pressure, the respiration gave evidence of failing and it became necessary at *e* to give artificial respiration. At this time the inflow rate rapidly increased.

In this experiment the variations in constrictor tone can be regarded as secondary to the effects on the center of the changes in arterial pressure. It is obvious that the diminished peripheral tone is not the cause, or at least not the sole cause, of the low arterial pressure; for if it were, the arterial pressure at 4:15, *e*, when the inflow rate was nearly the same as at the opening of the experiment, should have been up to the initial level. The fact that it was not presumably finds its explanation either in a diminution in the effective blood supply or in cardiac weakness.

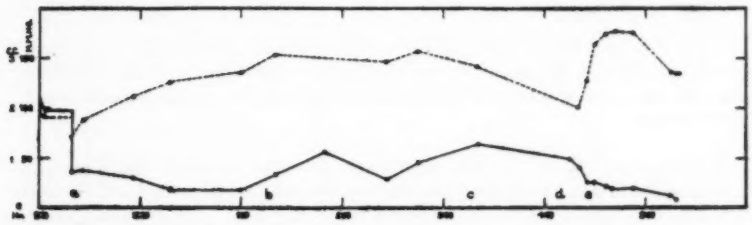

Fig. 1. Experiment 4. Temporary partial occlusion of the inferior vena cava in the thorax. Arterial pressure, —●—●—●—; femoral inflow, -●-●-●-. *a*, cava occluded; *b*, cava opened; *c*, thorax closed, artifical respiration off; *d*, respiration bad; *e*, artificial respiration.

In *experiment 20* (*fig. 2*), intestinal and hepatic inflow determinations were made. As the latter were unsatisfactory, no further reference need to be made to them. The vena cava at first was so occluded, at *a*, as to lower the arterial pressure to 82 mm. Hg., and then for 2 hours, *b*, to 34 mm. Hg. With deocclusion, the arterial pressure rose in the course of about 20 minutes to 100 mm. Hg., but it then fell and reached the level of 50 mm. Hg. about 15 to 20 minutes later. Later the heart stopped suddenly, as though the ventricles had fibrillated. Within a few minutes after beginning the more complete occlusion, it was found, *c*, that the ether could be dispensed with, and it was not necessary to administer it again.

After determining the normal inflow rate, inflow readings were not again made until the vena cava was deoccluded. The first reading after the period of occlusion was at the low limit of the initial range; but three subsequent readings, the last of which was made while the pressure was at 50 mm., Hg., were at the upper limit of the initial readings. With death there was some further increase in the inflow rate, followed by a steady decrease (constriction of the larger arteries?).

The intestinal inflow readings in this case show that after deocclusion the center has lost much of its activity; for in the presence of a very low arterial pressure, which ordinarily would have constituted a very strong stimulus, the center is not able to maintain more than the normal grade of arterial constriction.

They also show that the center possesses some residual tone up to the moment of death.

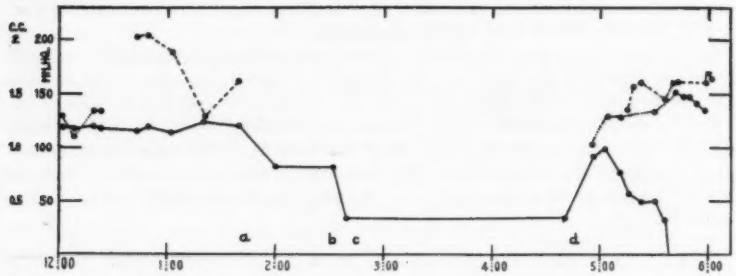

Fig. 2. Experiment 20. Temporary partial occlusion of the inferior vena cava. Arterial pressure, —●—●—●—; hepatic inflow, – – ●– – ●– –; intestinal inflow, .. ●.. ●.. ●... *a*, cava clamped; *b*, cava clamped more; *c*, ether discontinued; *d*, unclamped.

Experiment 50 (*fig. 3*). In this experiment the occlusion was of such a grade as to reduce the arterial pressure from 120 mm. Hg., to, for the most part, 40 to 60 mm. Hg., and was continued for 2 hours 8 minutes, *a* to *b*. The arterial pressure obtaining during occlusion in this experiment was much higher, therefore, than in experiment 4 (fig. 1) and somewhat higher than in experiment 20 (fig. 2). During the period of compression the femoral inflow rate decreased during about 40 minutes, eventually falling below the lowest of the initial readings; it then steadily increased so that at the time of deocclusion it was decidedly above normal. In this stage of the experiment we again see, first, the effort of the vasoconstrictor center to compensate the low pressure, and then a loss in the tone of the center. The latter effect may be due either to fatigue, or to functional incapacity of the center from the diminished blood supply, or to functional incapacity of the peripheral mechanism, also resulting from the reduced blood flow. Reasons for discarding the last explanation will be presented later. But whatever its cause, the loss of function at this time is not complete, as is demonstrated by the further increase in inflow rate that occurs at the close of the experiment.

With occlusion of the cava, the portal pressure rose at once from 8 mm. Hg. to 12 mm. Hg., but then fell promptly to 10 mm. Hg. This level was then maintained, possibly falling a bit toward the close of the period, as the arterial pressure declined. It is interesting to note how constant the portal pressure remains, varying not more than 1 mm. Hg., while the arterial pressure falls from 73 to 40 mm. Hg., and while the vasomotor tone (splanchnic, presumably, as well as somatic) swings from well above normal (11:30 to 11:40) to well below normal (1:00).

There can be no doubt but that the arterial pressure falls when the cava is occluded because of the accumulation of the blood in the peripheral veins and capillaries. This experiment shows that a degree of occlusion of the cava that causes a rise of only 2 mm. Hg. in the portal pressure (a corresponding, and prob-

ably greater rise in pressure occurs also in the systemic veins peripheral to the obstruction (2)) is all that is necessary to cause an accumulation of blood in the veins of sufficient magnitude to lower the arterial pressure to 60 mm. Hg.

The jugular pressure (one reading only) also was increased slightly (1.2 mm. Hg.) by the occlusion. In view of the fact, just commented upon, that back pressure on a central vein produces very marked effects even when the rise in venous pressure caused thereby is slight, this rise may mean that the heart here is suffering from the effects of a deficient coronary circulation.

Upon deocclusion, the arterial pressure rose at once from 34 to 65 mm. Hg., then more slowly (in about one-half hour) to 80 mm. Hg., after which it began slowly to sink (until 2:30). The femoral inflow during this phase of the experiment, continued to increase for about 10 minutes and then progressively decreased until 2:30. The portal pressure actually rose momentarily upon deocclusion and then sank progressively, until by 2:30 it was only 6 mm. Hg.,—that is, below the normal of 8 mm. Hg. The jugular pressure shows a further slight, and possibly, insignificant rise in this phase of the experiment.

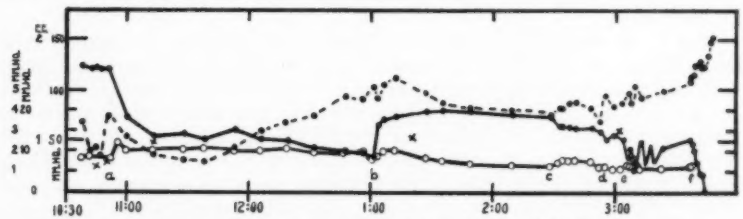

Fig. 3. Experiment 50. Temporary partial occlusion of the inferior vena cava. Arterial pressure, —●—●—●—; portal pressure, —○—○—○—; jugular pressure, —x—x—x—; femoral inflow, – –●– –●– –. *a*, cava clamped; *b*, clamp removed; *c*, slow asphyxiation; *d*, fresh air restored; *e*, temporarily clamped cava; *f*, clamped trachea.

The increase in vasomotor tone, the rise in arterial pressure and the fall in the portal pressure seen in the phase beginning some 10 minutes after deocclusion, is a combination that is easily accounted for; it is merely necessary to assume that the volume of blood liberated by deocclusion permits the heart to raise the arterial pressure. This soon improves the condition of the vasomotor center, and the peripheral resistance consequently increases. The fall in portal pressure is then explained by this increase in the peripheral resistance. Reduced vasomotor tone is not the whole trouble, however; for despite a peripheral resistance that is only a bit below the initial range, the arterial pressure is far below normal.

In the last phase of this period (1:45 to 2:30), despite the continued increase in the peripheral resistance and a further decrease in portal pressure, the arterial pressure falls. Failure of the heart or a diminution in the effective volume of blood alone could bring about this combination of events. The same inference must be drawn from the fact that, although at the end of this phase vasomotor tone lies within the normal range (though at the upper limit, to be sure), the portal and arterial pressures are well below normal. Inasmuch as the jugular

pressure continues to increase, even though slightly, during this phase of the experiment, the possibility must be entertained that failure of the heart is partly the cause of the fall in arterial pressure.

At this point, *c*, the animal was made to rebreathe the air contained in a 20 liter tank. The vasomotor tone, for some unknown reason, at first decreased, but it then increased, presumably through the action of the asphyxia that eventually developed. While the vasomotor tone was decreasing, the arterial pressure fell and the portal pressure rose. This is exactly the complex a vasodilatation should produce. But although, during the subsequent increase in vasomotor tone, the portal pressure fell, the arterial pressure, instead of rising, fell still further. This behavior of the arterial pressure possibly is to be attributed to the action of the asphyxia upon the heart.

With the cessation of rebreathing, *d*, the peripheral arteries dilated markedly. The arterial pressure at the same time fell, while there was but little change in the portal pressure. By this fall the arterial pressure was carried down to the level of 50 mm. Hg. and, excepting a slight momentary recovery, it did not again get above that level. At this time, vasomotor tone and portal pressure both were below normal.

Now, *e*, the effect was determined of momentarily clamping the inferior vena cava. When the clamp finally became tight the vasomotor tone diminished sharply, the arterial pressure fell sharply, and the portal pressure rose sharply, though slightly. At the same time the respiratory center failed and the clamp was removed and recourse had to artificial respiration. Within a minute or two the respiratory center became spontaneously active again and artificial respiration was discontinued, the vasomotor tone improved, the arterial pressure rose, and the portal pressure sank. This procedure was tried a second time with the same results, at least, in so far as the arterial pressure and the respiratory center are concerned (venous pressure and inflow readings were not made).

It will be noted that the effect of clamping here is very different from what it was at the beginning of the experiment; instead of causing arterial constriction it causes dilatation. This result presumably is to be explained by the nearness of the medullary centers to the limit of their viability; interference with the circulation, instead of causing them to respond as they do usually, with increased activity, lowers their activity. Throughout this stage of the experiment the general trend of the vasomotor tone was downward, while the arterial pressure and the portal pressure, essentially, were stationary. The animal was then killed by clamping the trachea, *f*. The arterial and portal pressures fell promptly, while the inflow rate was further accelerated. The jugular pressure (5 estimations) increased slightly throughout the experiment. Toward the end of the experiment, only the lightest anesthesia was needed to keep the animal from moving.

Experiment 45 (*fig. 4*) is described because it presents some new points of interest and also throws light on some of the events of the preceding experiment. It should be stated at the outset that this animal had very much enlarged thyroids and the enlargement of the carotid arteries often associated with that condition. It is possible that some of the unusual findings are referable to this fact. A general survey of the results of the experiment may be given before taking them up for discussion in detail.

Occlusion of the vena cava (at *a*, fig. 4) lowers the arterial pressure from 88 to 42, but eventually to about 30 mm. Hg. After 18 minutes, at *b*, respiration and heart fail. Through cardiac massage coupled with artificial respiration, the heart recovers and the circulatory changes then progress without any apparent break in continuity; but the respiratory center does not recover. Even before this failure of the respiration and the heart occurred it was realized that the caval occlusion, though carrying the arterial pressure no lower than it has in other experiments, was going to cause too profound a change in the circulation; therefore, as is indicated in the figure at *c*, the clamp was opened a bit. The arterial pressure rose slightly and then returned to its previous level. The effect of this partial release of the cava, though seemingly ephemeral, probably was long lasting; for it checked somewhat the rate of fall of the arterial pressure. At *e*, *f*, *g*, *h*, *i*, *j*, *k*, *l* and *m* the clamp was partially opened until the jaws were wide apart, whereupon the clamp was removed. Inasmuch as after *h* the arterial pressure failed to show even the temporary, slight elevation up to this time in evidence

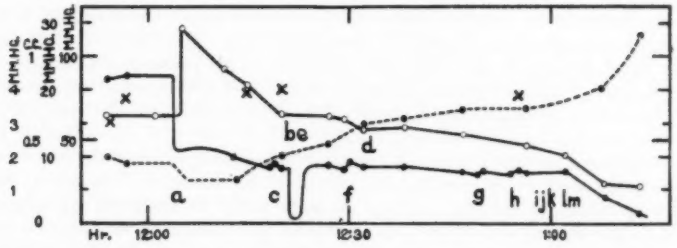

Fig. 4. Experiment 45. Temporary partial occlusion of the inferior vena cava (hypertrophy of thyroid). Arterial pressure, —●—●—●—; jugular pressure, x x x ; portal pressure, —○—○—○—; femoral inflow, – – ●– – ● – – ●– –. *a*, cava clamped; *c*, *e*, *f*, *g*, *h*, *i*, *j*, *k*, *l* and *m*, partial unclampings; *b*, heart and respiration stop; recovery by cardiac massage and artificial respiration.

with each partial decompression, it may be assumed that the clamp was then wide open. The fall in arterial pressure at the end, to all appearances, was due to failure of the heart. The jugular pressure, which was high at the outset, did not change materially during the course of the experiment.

The portal venous pressure, also, was high at the start (16 mm. Hg.) and was markedly increased by compression of the cava, reaching the astonishing value of 29 mm. Hg.; but it then gradually fell and in 17 minutes was back practically to its original level. It then fell more gradually until failure of the heart caused the arterial pressure to fall rapidly, when the portal pressure likewise fell more rapidly.

The femoral inflow rate, as is always the case, at first diminished upon obstructing the cava. It is quite likely that, had a reading been made immediately after applying the clamp, a still slower inflow rate would have been found. The inflow rate then rapidly increased, after a time, however, at a diminishing rate. Eventually, when the heart finally failed, the inflow rate again accelerated.

We may now attempt an interpretation of these results. The damming back of blood by occlusion of the cava lowers the arterial pressure. The vasomotor center at first is stimulated by the anemia and constriction results. Despite this constriction, however, the arterial pressure is not raised and the anemic center begins to lose in activity.

An interesting stage of the experiment is where the vasomotor tone, while diminishing, crosses the normal level (at about *c*); for it so happens that at the same moment the portal pressure and the jugular pressure likewise are at their initial levels; the arterial pressure, though, is subnormal. For estimating the efficiency of the heart at this time the behavior of the jugular pressure, as has been said, is of but little assistance. While it is true that the heart stopped at *b*, it is to be presumed that this stoppage was secondary to failure of the respiration; for after resuscitation of the animal by cardiac massage and artificial respiration, the heart continued to beat, whereas the artifical respiration had to be continued during the rest of the experiment. The lowness of the arterial pressure must therefore be attributed either to accumulation of blood in the veins and capillaries or to extravasation. The conditions here were unusually favorable for the development of both of these processes. The high portal pressure would favor distension and filtration, while the slight pressure gradient in the splanchnic capillaries (difference between arterial and portal pressures), amounting for the most part to from 13 to 18 mm. Hg., only, would favor the transudation that comes with stagnation of the circulation. The brevity of the period, as will be made clear in another connection, does not invalidate the latter explanation.

The heart, started by massage and supplied with blood now aerated by artificial respiration, is able to continue its work about 40 minutes longer, when, owing to the continued low and falling arterial pressure, it again fails. At the time the respiratory center fails, *b*, the tone of the vasomotor center, it will be noted, is only a little below normal. In order to make sure here (at *d*) that the respiratory center had actually failed and was not merely in a state of apnoea, artificial respiration was discontinued for a period of 80 seconds: the animal made not the slightest effort to breathe. This observation confirms the well-known fact that the vasomotor center is one of the most resistant of the bulbar centers to cerebral anemia (3). The vasomotor center continues to lose in tone, though before the heart finally fails, the tone tends to strike a level. During this period, the portal pressure falls more rapidly than the arterial pressure, while the jugular pressure shows no material change. This combination of circumstances can scarcely be ascribed alone to increasing incapacity of the heart, nor can it be attributed to the diminishing vasomotor tone, for it is inconceivable that this could cause the portal pressure to fall more rapidly than the arterial pressure. Diminution in the effective volume of blood, therefore, again seems to be the sole factor accounting satisfactorily for the loss, in large part at least, in the efficiency of the circulation in this stage of the experiment. This is all the more remarkable because it is in this stage that the removal of the caval clamp ought to have made possible the return to the general circulation of some of the blood which had been trapped in the veins. The question arises, why does not the blood thus released become effective? Has it disappeared from the blood vessels, or has an increase in the capacity of the arterioles and capillaries through loss in vasomotor tone, and also of the veins, caused it to disappear? These are questions that must

be left unanswered. But whatever may be the explanation of this interesting circulatory state, it is to be noted that despite the early complete failure of the respiratory center, the vasomotor center possesses, until the heart fails, the capacity of holding the inflow rate well below that permitted into the unbridled arterioles.

Finally, we wish to call attention to the fact that but for the failure of the respiratory center, the condition to which this animal was reduced resembled typical shock; ether was no longer necessary, and the arterial pressure was below 50 mm. Hg. The grade of vasomotor depression that obtained before the heart finally began to fail, probably would not of itself have brought on shock; presumably it required this, plus the mechanical disturbance in the circulation that was caused by occlusion of the vena cava. However this may be, it is obvious that this animal was more susceptible to the effects of caval occlusion than any of the other animals thus far considered; and that the grade of circulatory inefficiency which, in other animals treated in the same way, does not supervene until some time after deocclusion, in this case develops while the cava is being occluded. We will have occasion to refer to this subject again in connection with the next experiment.

Experiment 47 (*fig. 5*). We describe still another of this series of experiments because of its interest in several respects. For some unknown reason this animal, like the preceding one, had unusually high initial jugular (3.0 to 3.5 mm. Hg.) and portal (14 to 15 mm. Hg.) pressures; and, as in the preceding case, it was necessary to open the clamp early in order to keep the animal alive. When the cava was so clamped (at *a*) as to carry the arterial pressure from the initial value of about 140 mm. Hg., down to 46 to 40 mm. Hg., the jugular pressure was scarcely affected, whereas the portal pressure, exactly as in the preceding experiment, rose immediately to 27 mm. Hg. and then slowly fell. The rate of femoral inflow decreased immediately and then slowly increased. At *b*, just 20 minutes after applying the clamp, the heart became irregular and, lest it might stop, the clamp was opened somewhat. A tremendous slowing in heart rate and increase in pulse amplitude resulted; the arterial pressure rose and the portal pressure fell. Despite this improvement in the circulation, and despite the fact that the occlusion in this experiment had not been any more severe than usual and had not lasted nearly as long as usual, the animal stopped breathing (at *c*). Artificial respiration was then started, without ether; within a few minutes the heart-beat improved and the clamp was then tightened a bit. The animal now began to breathe spontaneously and artificial respiration was discontinued (at *d*); but 5 or 6 minutes later (at *e*) it became necessary to resume artificial respiration. The arterial pressure soon fell below 40 mm. Hg.; so the clamp was gradually opened, and though by 12: 18 (at *f*) it was wide open, the circulation did not improve.

The inflow rate gives the clue to at least one of the causes of the circulatory failure. The estimation made a few minutes after the first failure of the respiration, *c*, showed that the peripheral resistance was well below the lower limit of the initial estimations; and subsequently a very marked further dilatation dedeveloped. Evidently both centers, respiratory and vasomotor, had become inefficient.

Even though the cava was now wide open, the arterial and portal pressures continued to fall, and as death seemed imminent a large dose of adrenalin (1 cc. of 1: 1,000) was given intravenously (at *g*). The arterial pressure rose at once to 234 mm. Hg., indicating that the heart was still capable of as much exertion as in a normal animal. The inflow determinations show that the rise of pressure was due to a peripheral constriction which practically amounted to complete closure of the arteries. But within 18 minutes (12:45) the arterial pressure had fallen to 45 mm. Hg., despite a peripheral constriction that was still almost complete. The jugular pressure, which had mounted about 0.5 mm. Hg. while the circulation was failing (possibly an effect of the artificial respiration), after the administration of the adrenalin fell 1.5 mm. Hg., possibly as a result of some action of the adrenalin upon the heart, or of the removal of the *vis a tergo* through closure of the arterioles. The portal pressure, which had fallen to 5 mm. Hg., was raised by the adrenalin injection to 15 mm. Hg., but fell again and by 12:45 was at 7.5 mm. Hg., a usual value in normal animals. At this particular moment, therefore, despite normal jugular and portal pressures, despite a heart that is capable of elevating arterial pressure quite as high as can a normal heart, and despite a high peripheral

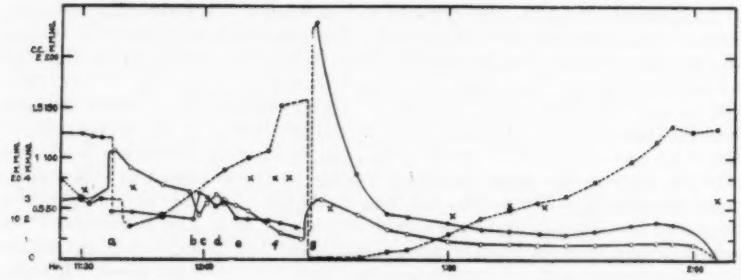

Fig. 5. Experiment 47. Temporary, partial occlusion of the inferior vena cava. Arterial pressure, —●—●—●—; jugular pressure, x x x ; portal pressure, —○—○—; femoral inflow, – –●– –●– –. *a*, cava clamped; *b*, partial unclamping; *c*, respiration stops, artificial respiration started; *d*, artificial respiration discontinued; *e*, artificial respiration resumed; *f*, clamp being gradually opened, at *f*, is wide open; *g*, 1 cc. 1: 1000 adrenalin intravenously.

resistance, the arterial pressure is insufficient to maintain life. At this time the respiratory center was still inactive and it may be surmised that the vasoconstrictor center also was inactive or, at least, depressed. It is conceivable, therefore, that while the adrenalin was constricting the arterioles in certain parts of the body, in others the vessels were still maximally dilated. But experiments to be described in another connection, show that the vessels of both the somatic and splanchnic areas are constricted by these large doses of adrenalin. It does not seem possible, therefore, that in the areas not acted upon by adrenalin the resistance would be low enough to permit of as large a fall in arterial pressure as is here seen. Consequently there is left but one other factor upon the basis of which it is possible to account for the failure of the circulation, namely, an insufficient effective volume of blood.

Summary and discussion

The experiments that have been cited as illustrative of the results obtained after partially obstructing the cava can be divided into two groups upon the basis of the rate of onset of circulatory failure. In one group the circulatory failure comes on some hours after deocclusion; in the other, much earlier, it may be while the cava is still occluded.

The results obtained in the *first group* may be generalized and interpreted as follows:

While the cava is occluded the rise in venous pressure causes blood to accumulate in the veins, both systemic and portal, and possibly in the capillaries also, and the arterial pressure consequently falls. The vasomotor center, stimulated by the anemia thus produced, immediately calls forth a peripheral constriction; but later the anemia begins to tell on the center and its tone begins to give way. The moment at which this change takes place can often be recognized: while the tone is increasing the clamp on the cava must be tightened more and more in order to hold the arterial pressure down, but while the tone is diminishing it becomes necessary to gradually loosen the clamp in order to prevent the pressure from falling too low. The rise in portal pressure (the pressure in the systemic veins peripheral of the clamp has not been measured) caused by the occlusion which leads to the sequestration of blood may be surprisingly small and may be taken to indicate how small is the resistance that is needed in order to cause a marked diminution in the effective volume of blood. The effective blood pressure, that is, the difference between the arterial and venous pressure, with respect to that obtaining in the second group of cases, is relatively large; the flow of blood, therefore, is relatively free.

When the cava is unclamped in those instances in which the fall in pressure begins fairly promptly (within a few hours), the arterial pressure rises abruptly at once and then more and more slowly, it may be, to attain a height of 80 or 90 mm. Hg. or more; but sooner or later, usually in less than an hour or two, the pressure starts on the decline that leads to death in the course of some hours. When the onset of the shock-like failure of the circulation is longer delayed, the arterial pressure upon unclamping the cava not infrequently, at first, ascends gradually to a level as high as, or even higher, than that obtaining before the clamp had been applied. The systemic venous pressure may rise slightly throughout the course of the experiment; and this rise may be indicative of some growing inefficiency of the heart (see below).

The portal pressure may be slightly above normal for a while after deocclusion, but it returns to, and then falls below, normal, it may be before the arterial pressure begins to give way. These changes in portal pressure obviously are modified somewhat by the coincident behavior of the peripheral resistance.

With deocclusion and the consequent improvement in conditions, the tone of the vasomotor center increases until, often, the inflow reaches the upper limit of normal. Unfortunately we have not made inflow observations in any of the instances in which the arterial pressure mounts above its pre-occlusion level and, therefore, do not know the condition of the peripheral resistance during the interesting stage of high arterial pressure. The vasomotor tone often continues to increase after the arterial pressure begins to fall, but eventually it begins to slowly decrease. This decrease presumably is to be attributed in part to the continuance of the animal on the table and in part to the diminishing supply of blood to the brain determined by the falling arterial pressure. After the arterial pressure has fallen to 50 mm. Hg., or thereabout, the tone of the center and the arterial pressure usually start on a more rapid decline. There almost always is some, indeed, often considerable, residual vasomotor tone up to the moment the animal dies.

We here point out that if, in an otherwise normal animal, the arterial pressure were lowered by some means to the level to which the arterial pressure rises shortly after deocclusion (often less than 80 mm. Hg.), a very marked constriction unquestionably would result. The fact, therefore, that the center under this low pressure does not succeed in increasing vascular tone beyond that which is normal for a normal arterial pressure, indicates that the center is depressed. Under the stimulus of the continuous low arterial pressure it cannot effect more than a normal grade of constriction. This is not due to the fact that the effort of the center at this time is maximal; for if the animal is asphyxiated, some further increase in tone can be elicited, but this response is not nearly so vigorous as is that given by a normal animal.

Although impairment of the vasomotor center undoubtedly is a factor in the failure of the circulation in this group of cases, it by no means is the only factor. There is evidence that the heart also suffers to a certain extent. In a long series of experiments of the same general character as those described in the present paper, but performed with another object in view, impaired heart action was not infrequently encountered. In such instances, at some time during the period of caval occlusion the heart became irregular in force and rhythm and sometimes stopped

despite removal of the caval clamp. When, in such cases, the heart did not stop and it was possible to carry the animal through the period of occlusion without a return of the cardiac symptoms, it was found that the average height attained by the arterial pressure after removing the clamp was lower than in the cases that had not exhibited these cardiac symptoms. This failure of the pressure to attain an average height during the post-occlusion period presumably is indicative of cardiac weakness. Whether the heart suffers as well in cases in which, during the clamping period, it does not become irregular, is a question we are not prepared to answer. The fact that in these cases adrenalin may raise the arterial pressure quite high does not necessarily mean that the capability of the heart is unimpaired. This is a question that could be answered only by determining the way the heart would hold up under a long continued high resistance.

In the description of the individual experiments, we have, in every instance, called attention to unmistakable evidence of a reduction in the effective blood volume. The blood supplied the heart did not seem to be sufficient in amount to permit that organ to maintain the pressure in the aorta. The condition of the veins gives no definite clue to the whereabouts of the blood that is out of circulation; if it is on the venous side of the circuit it is so held there that it does not materially increase the venous pressure.

The *second group* includes only two cases, both of which had abnormally high initial venous pressures, both systemic and portal. In both, occlusion of the cava caused some further elevation of the systemic, and an enormous elevation of the portal venous pressure. This state of affairs had the effect of markedly reducing the effective blood pressure and, therefore, the blood flow, especially, presumably, in the splanchnic area. The early failure of the respiratory center and the early turning of the preliminary vasoconstriction into a rapidly developing vasodilatation in both cases, and the temporary failure of the heart in one of them, all, presumably, are indicative of a blood supply to the tissues deficient out of all proportion to the arterial pressure of the clamping period. It is barely possible that this is to be attributed to the influence the unusually high venous pressure, exhibited by these two animals, must have had upon the effective arterial pressure. But these direct effects of the deficient blood supply to the tissues are not the sole cause of the failure of the circulation. For in both of the experiments there was a time (some 15 to 17 minutes after applying the clamp) when the heart was still efficient, when the vasomotor tone had not yet passed

below the normal range, and when the venous pressure (portal at least) had returned to its initial level; yet the arterial pressure was far below normal. Evidently a reduction in effective blood volume must be the factor that accounts for the low arterial pressure. This reduction in effective volume could not have been the result merely of static distension of the veins, because the venous pressure, in certain periods, was not any higher than the initial pressure. Loss of tone as a result of some action on a veno-pressor mechanism such as has been described by Hooker (4), loss of tone as a result of local nutritional disturbances, and concentration of the blood are other possible explanations. But further than to state that as rapid a concentration of the blood as would be necessary to effect a change of sufficient magnitude to cause the circulation to fail in 17 minutes, in our experience is not entirely without the realm of possibility, we would postpone the discussion of this question until certain other experiments bearing on the subject have been presented.

Pathological picture and its significance

In this connection the anatomical picture presented by these cases is of some significance. At autopsy the abdomen, in perfectly successful cases, contains no fluid. The small intestine often contains a little blood and the mucosa is usually of a deep bluish-red, almost hemorrhagic, color. This appearance is usually most marked in the upper parts of the small intestine. Below the ileocecal valve the congestion usually is not nearly so striking and may be entirely absent. The spleen often is enlarged and may contain hemorrhagic areas, though it may show no gross changes whatever. The liver as a rule does not seem to be enlarged nor especially full of blood, though Janeway and Jackson (2) state that the volume of the liver, measured plethysmographically, increases, at least until the animal dies. We have carefully weighed the liver in nine of our cases and have found the average to be 3.93 per cent of the body weight. This figure is at the lower limit of normal (3.85 to 5.9 per cent), as given by Ellenberger and Baum (5). Indeed, of the nine livers, seven actually were at or below Ellenberger and Baum's lowest figure, while none reached the uppermost figure. Microscopically, in the few livers we have examined, the venules do not seem to be especially full of blood. This is sometimes true also of the spleen, though more often it is the seat of hemorrhages. The most constant and the most striking change, though, is found in the intestine; almost invariably the capillaries and the venules of the villi are enormously

distended and solidly packed with red corpuscles. The appearance is quite the same as that presented by the intestines of animals dying of shock from exposure of the intestines (1).

This picture indicates that a part of the blood, or at least, of the blood corpuscles, is retained in the capillaries and venules of the villi of the intestines, and sometimes, a part in the spleen. If any excess is retained in the liver, kidneys or stomach, gross and microscopical examination usually fails to reveal it. Other organs have not been carefully examined. The significance of the amount of blood retained in the capillaries and venules will be considered in another connection.

In their experiments Janeway and Jackson found that the volume of the intestine, measured plethysmographically, did not increase, whereas that of the liver did, and they conclude that "there is certainly no special sequestration in the vessels of the small intestine. The experiments indicate rather that there is a special sequestration of the blood in the capillaries of the liver." Whether they made anatomical and histological examinations in addition to the plethysmographic observation is not stated. It is here, however, that our results are not in agreement with theirs. The plethysmograph in their hands has failed to show the concentration of the blood in the villi of the intestines, which we find almost invariably; has led them to conclude that the blood is sequestered in the capillaries of the liver, while we, as a rule, find no post-mortem increase in liver weight or distension of liver blood vessels; and has failed to show the loss of vasomotor tone, which in our experience is characteristic of all excepting the first stages of these experiments. We do not mean to say that the volume of the liver is not increased during life, but merely that the blood is not "*sequestered*" there; otherwise the weight of the liver would be increased and the capillaries distended post-mortem. Nor do we mean to say that the volume of the intestine does not decrease; but merely that a decrease in the volume of a hollow and muscular organ does not necessarily mean that there has been a diminution in the volume of blood its vessels contain. It is quite conceivable that the immediate vasoconstriction and fall in arterial pressure which occur upon clamping the cava decrease the intestinal volume, and that this decrease might be maintained throughout the rest of the experiment; the subsequent diminution in vasomotor tone failing to manifest itself as an increase in the caliber of the vessels, because of the falling arterial pressure and because of the falling venous pressure; the volume effect of the accumulation of blood in the capillaries and venules, being compensated in part by the disappearance of plasma and in part by the emptying of the other vessels of the bowel.

SHOCK BY TEMPORARY PARTIAL OBSTRUCTION OF THE AORTA

Obstruction of the inferior vena cava acts detrimentally upon the circulation in several ways, presumably, *a*, by mechanically causing blood to accumulate in the veins and capillaries, thus removing it from effective circulation; through asphyxial damage, *b*, to the walls of the veins and capillaries, *c*, to the heart and *d*, to the medullary centers. In an effort to determine the relative importance of these several possibilities we have studied the consequences of temporarily interfering with the blood supply to a large part of the body by another procedure, —namely, partial occlusion of the thoracic aorta. For this purpose the aorta was occluded in the chest just beyond the origin of the left subclavian artery. In these experiments, therefore, the blood supply to the upper parts of the body,—the medullary centers, the heart, etc.,—is not diminished; and there is no increase in back pressure in the veins. It was hoped that by thus dissociating the several factors the desired information might be gained.

Methods

The pressure was followed in the femoral artery as well as in the carotid, and the aorta so occluded that, in the case of the three experiments we have done, the femoral pressure at first fell to 45, 30 and 31 mm. Hg., but eventually, by the end of the clamping period, to 40, 16 and 15 mm. Hg., respectively. In two of the experiments therefore, the peripheral pressure for part of the time was held much lower than in the caval experiments. The period of occlusion lasted, as in the caval experiments, about 2 hours. It was necessary, of course, to give artificial respiration throughout the experiments. All three animals behaved essentially alike, yet the differences that occurred are so interesting that each experiment may profitably be considered separately, before an attempt is made to generalize.

Experiment 35 (*fig. 6*). Partial occlusion of the aorta, *a*, raised the carotid pressure from the normal of 90 to 130 to 140 mm. Hg. The increased blood supply to the brain thus effected, at first decreased the vasomotor tone, but then the latter immediately began to increase and before the end of the period of occlusion it had returned to normal.

With deocclusion, *b*, the arterial pressure fell at once to about the initial level and then slowly declined to attain (after 1 to 1½ hours) a pressure of 50 mm. Hg., though the pressure did not remain consistently below 50 mm. Hg. until over 2 hours had elapsed. The vasomotor tone increased sharply at first and then more slowly, so that an hour after deocclusion it was definitely above normal. In the

next period, 2:40 to 3:40, the arterial pressure ranged irregularly between about 40 and 60 mm. Hg. At the same time the inflow rate also fluctuated considerably, around, or somewhat below, the normal level. In the last stage, beginning at about 3:40, the arterial pressure slowly declined from about 50 to 36 mm. Hg., when heart failure supervened. The vasomotor tone in this stage diminished quite rapidly, reaching, to be conservative, the upper limit of normal at the time the heart failure and death led, after a slight asphyxial constriction, to complete failure of the vasomotor center. The animal required no ether during the last 40 minutes of the experiment. The eye reflex was not clearly obtainable.

At 4:00, *c*, the reactivity of the vasomotor center was tested by stopping the artificial respiration. Some constriction did occur. The heart then stopped, but was revived by massage, the artificial respiration being resumed at the same time.

After deocclusion, therefore, the arterial pressure falls despite increasing peripheral constriction. The low arterial pressure presumably at first causes this

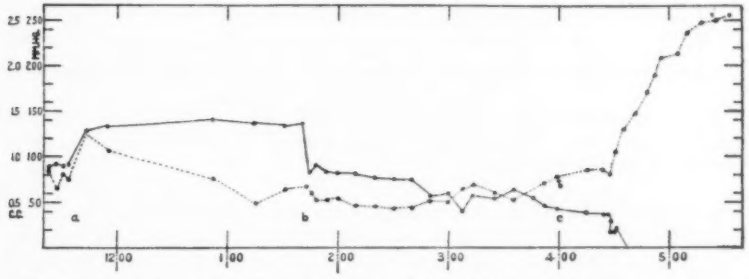

Fig. 6. Experiment 35. Temporary, partial occlusion of the aorta. Carotid pressure, —●—●—; femoral inflow, – – ●– – ●– –. *a*, aorta clamped; *b*, aorta unclamped; *c*, animal temporarily asphyxiated.

constriction through stimulation of the center. The subsequent diminution in peripheral resistance presumably is attributable to the giving way of the centers as a result of the continuance of the low arterial pressure.

In *experiment 36* (*fig. 7*), occlusion of the aorta, *a*, raised the carotid pressure from 102 to a maximum of 146 mm. Hg. by the end of an hour. The carotid pressure then steadily declined, despite the fact, previously referred to, that the femoral pressure steadily decreased. This decline, as well as the fall in femoral pressure, seems to be explained by the behavior of the vasomotor center; for the tone of this center is decreased not alone immediately upon occluding the aorta, as in the preceding experiment, but in addition, the tone decreases more or less consistently and markedly during the whole of the occlusion period.

With deocclusion, *b*, the arterial pressure falls to about 60 mm. Hg., where it remains for a period of 1 hour and 40 minutes (until 2:10). The inflow rate decreases sharply at first and then more slowly, eventually coming into, or slightly below, the initial range. In the last stage, beginning at about 2:10, the arterial

pressure falls to 50 mm. Hg. and then well below it. Vasomotor tone diminishes slowly at first and then more and more rapidly. The animal was finally killed by stopping the artificial respiration, *c*.

In this experiment, therefore, the inflow rate did not become faster than normal until the arterial pressure had been below 50 mm. Hg. for some time. The animal required no ether during the last hour. The eye reflexes were present practically to the end. The behavior of the arterial pressure and of the inflow rate during the period of deocclusion is so similar to that of the preceding experiment that no additional comment is necessary.

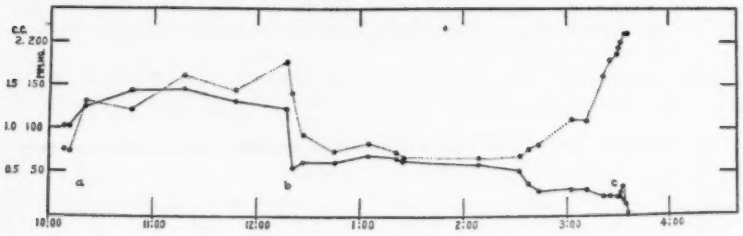

Fig. 7. Experiment 36. Temporary, partial occlusion of the aorta. Arterial pressure, —●—●—; femoral inflow, – – ● – – ● – –. *a*, aorta clamped; *b*, aorta unclamped; *c*, asphyxiation.

In *experiment 52* (*fig. 8*), clamping the aorta (at *a*) raises the arterial pressure from 105 to 110 to 145 mm. Hg. in the course of about 50 minutes. The pressure then declines until, just before deocclusion, it measures 80 mm. Hg. The general trend of the femoral inflow rate parallels the arterial pressure; but the short, sharp fluctuations of the inflow rate are opposite in direction to those of the central arterial pressure. The general trend, presumably, is an expression of the effect upon centers of the improvement of the circulation through them, while the sharper changes, presumably, are referable to spontaneous changes of some kind in the activity of the vasomotor center, vasodilatation or vasoconstriction being accompanied by a fall or a rise in pressure, respectively. By the end of the occlusion period (at *f*) the inflow has returned almost to the initial rate, despite the fact that the arterial pressure is now well below normal. This fall in arterial pressure, therefore, is not referable to a vasodilatation.

Inasmuch as the circulation through the heart is improved by the rise in pressure in the aorta, weakness of the heart muscle cannot be invoked as a cause of the fall in pressure. It is possible, though, that the high pressure the heart had to work against may overtax its capacity, and cause some inefficiency through dilatation. A fall in arterial pressure due to dilatation of the heart should be associated with a striking rise in the jugular pressure. As a matter of fact the jugular pressure is increased, though somewhat less than 1.5 mm. Hg. The jugular pressure, however, does not increase as the arterial pressure falls, to the contrary, it actually falls somewhat. The fall in arterial pressure cannot, therefore, be explained upon the basis of an increase in the tax on the heart; rather, the fall

in arterial pressure actually relieves the heart. The portal pressure, as might have been anticipated, is lowered by the aortic obstruction, and remains low, with unimportant fluctuations, throughout the period. As, therefore, at the close of the occlusion period the peripheral resistance is practically normal, and as the efficiency of the heart is not impaired, the low arterial pressure can be accounted for only by a diminution in the effective volume of blood.

If occlusion of the aorta here works injuriously to the circulation by damaging the parts in which the blood flow has been cut down, removing the obstruction should increase still further the difficulty of maintaining the circulation. This is exactly what happens: with deocclusion the arterial pressure at once drops below the level of 50 mm. Hg. and continues to fall, the animal dying 15 minutes later through failure of the heart.

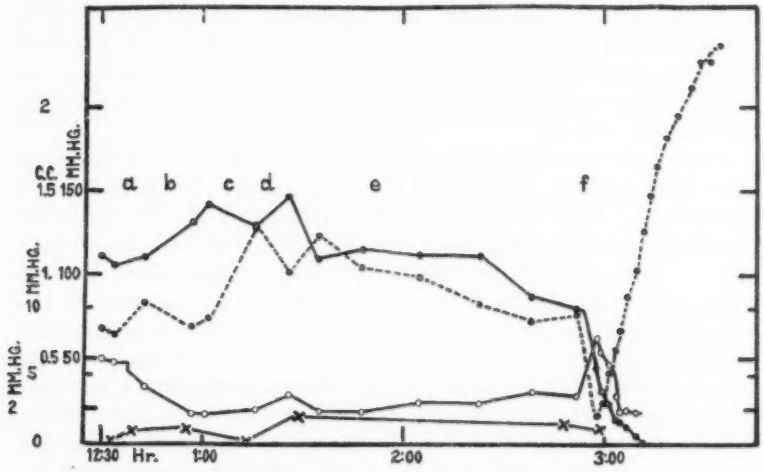

Fig 8. Experiment 52. Temporary, partial occlusion of the aorta. Carotid pressure, —●—●—; portal pressure, —○—○—; jugular pressure, —x—x—; femoral inflow – – ● – – ● – –. *a*, aorta clamped; *b*, clamp tightened; *c*, clamp loosened; *d* clamp loosened more; *f*, clamp off.

At the same time a tremendous peripheral constriction develops. Despite this constriction, which almost stops the inflow, the pressure in the arteries is only 45 mm. Hg. The fall in pressure cannot, therefore, be attributed to any inefficiency of the vasomotor mechanism either central or peripheral. This fact is of great importance in the interpretation of the results obtained; for although there is no reason for believing that the obstruction would injure the centers, it would not have been surprising if it had been found that the long lasting anemia had damaged the peripheral mechanism.

The extreme reduction in inflow rate gives way almost at once to a rapidly developing increase in rate which shows some further acceleration shortly after

the circulation stops. This increase in inflow rate unquestionably is attributable to failure of the center in consequence of the anemia associated with the failure of the circulation. It furnishes final proof of the previous perfect condition of the whole vasomotor mechanism.

With deocclusion, the portal pressure rose to 8 mm. Hg., and then fell as the arterial pressure fell. At no time was the portal pressure high enough to justify the slightest suspicion that an alteration in the portal circulation, through mechanical means, is the cause of the disturbance in the general circulation.

Discussion

The main results obtained in the foregoing experiments on occlusion of the aorta can be summarized in a few words. The fall of the arterial pressure to the level of 50 mm. Hg. is not due to inefficiency of the vasomotor mechanism, either central or peripheral, nor to inefficiency of the heart; it must therefore find its explanation in a reduction in the effective volume of blood.

We thus far have laid stress only upon the direct effects of the occlusion of the aorta as the cause of the failure of the circulation in these experiments. It is a well-known fact, however, that the circulation eventually will fail in animals merely kept on the table under an anesthetic. Thus, Morison and Hooker (6) show a record obtained in an experiment in which the animal, prepared by certain cannulations and by carefully removing from the abdomen one loop of the intestine and keeping this under salt solution, in which a pressure of 50 mm. Hg. was not reached until 11 hours had elapsed. The time required to lower the pressure to 50 mm. Hg. by mere exposure probably is shortened somewhat in our aortic experiments by the trauma of opening the thorax and of giving artificial respiration. The form of anesthesia also is a factor in the determination of the rate of onset of circulatory failure. Thus we have obtained evidence in the present experiments indicating that the administration of morphine increases very greatly the difficulty of bringing on the shock-like failure of the circulation. The morphine seems to have this influence indirectly through its action in sparing the ether necessary to maintain anesthesia. But all of these extraneous factors notwithstanding, there can be no question, in view of the very characteristic responses obtained, both physiological and anatomical, that failure of the circulation for the most part is referable to the effects of blocking the aorta.

Pathology

Out experience with aortic shock has been considerably more limited than with caval shock. But even this limited experience justifies the statement that the gross lesions found in animals dead of both types of shock are very much alike. In aortic shock the liver and kidneys show no certain changes; the stomach has occasionally shown some injected areas. The main changes, again, have been found in the intestines, which may contain bloody material, while the mucosa usually presents a bluish-red, hemorrhagic appearance, it may be, throughout the length of the small gut.

The microscopical picture also resembles very closely that seen after occlusion of the vena cava. Hemorrhages into the spleen pulp are sometimes to be seen. But the striking change is the tremendous distension of the capillaries and venules of the intestinal villi with solid masses of red corpuscles.

Comparison of caval with aortic shock, and discussion

In regard to the primary object of determining the effects of partial occlusion of the aorta it can now be stated definitely:

a. That exactly the same accumulation of blood in the venules and capillaries occurs after aortic occlusion as after caval occlusion; back pressure, therefore, is not the primary cause of the phenomenon, though, to be sure, there is the possibility that back pressure facilitates the process.

b. That failure of the circulation occurs as a result of aortic occlusion despite the absence of any evidences of damage to the heart such as seems, at times, to be caused by caval occlusion.

c. That in aortic occlusion the circulation fails despite (and contrary to what obtained in caval shock) a perfectly functionating vasomotor mechanism.

d. That, therefore, the accumulation and concentration of blood in the venules and capillaries and the failure of the circulation are primarily due to some local peripheral effect of the slowed blood stream.

The failure of the circulation in aortic shock and in caval shock, possibly also in intestinal shock, to us seems to be related to a process first observed by Mall and Welch. It is described by Welch in his classical article on "Embolism and Thrombosis" (7). When, in an animal, a mesenteric artery is partially or completely occluded, the smaller and the larger microscopic veins become more and more distended with red corpuscles and all of the phenomena of an intense venous hyperemia

appear. The red corpuscles accumulate in clumps or in solid columns. This change may become permanent, producing an evident obstacle to the forward movement of the blood. The same phenomena of distension with red corpsucles, clumping and stasis appear gradually in the capillaries. With the partial blocking of the venules and capillaries, red corpuscles begin to pass through the walls of these vessels by diapedesis; and after a time the hemorrhage becomes so great that it is difficult to observe the condition within these vessels. Mall and Welch found that the process begins to take place when the pressure in the artery is reduced to about one-fourth to one-fifth of the normal.

These are just about the conditions that obtain in our experiments during the period of aortic occlusion; and the similarity of the microscopical picture confirms the inference that the processes are similar in the two cases. Mall and Welch seem inclined to attribute the clumping of the corpuscles to the absence of pulsation. Their evidence does not, however, preclude mere slowing of the blood stream as a factor. If it is a factor, then a similar mechanism accounts for the condition found at autopsy in the capillaries and venules after partial occlusion of the cava.

The fact that after deocclusion both in cava[1] and aortic experiments, but especially in the former, the arterial pressure may rise to normal and the circulation show signs of failure only after some hours have elapsed, although explicable on the basis of asphyxial damage to vital functions, also can be accounted for upon the basis of the observation of Mall and Welch that the accumulation of corpuscles in clumps or in solid columns may become permanent.

The mechanism of the dilatation of the capillaries and venules is a question that has not been included in the scope of this investigation. It can, however, be assumed, on the basis of our observations, that the activity of neither the central nor the peripheral parts of the vasomotor mechanism need be affected in order that these processes may take place; the distention of the venules is not attributable to paralysis of nerve terminals. It, therefore, becomes highly improbable that failure of a veno-pressor mechanism (4) is responsible for the dilatation.

CONCLUSIONS

Properly graded temporary partial obstruction of the inferior vena cava above the liver or of the aorta beyond the origin of the left subclavian artery eventually results in the development of a condition closely resembling traumatic shock.

At the time the circulation gives evidence of failing the state of affairs is about as follows:

	AFTER CAVAL OCCLUSION	AFTER AORTIC OCCLUSION
Vasomotor mechanism...	Somewhat impaired	Efficient
Heart action.....	Possibly somewhat impaired	Unimpaired
Effective blood volume...	Evidences of reduction	Evidences of reduction

And at autopsy dilatation of the capillaries and venules of the intestinal villi by masses of red corpscles is found.

It is therefore concluded:

a, That the failure of the circulation after both manipulations is in part certainly due to the consequence of the sequestration of corpuscles in the capillaries and venules.

And *b*, that if back pressure in the veins, such as is produced by caval obstruction, is a factor in the development of this state of affairs in the capillaries and veins, it is not an essential one.

The fact that the vasomotor mechanism need not be involved (as for example in aortic obstruction) renders it unnecessary to invoke failure of a nervous veno-pressor mechanism in order to explain the fullness of the capillaries and venules. It can be explained upon the basis either of the change in the character of the blood stream, or of respiratory and nutritional changes in the walls of the vessels, or, and more probably, of both.

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GASTRIC RESPONSE TO FOODS[1]

III. The Response of the Human Stomach to Beef and Beef Products

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Among the foods of man the meats hold a high place. Because of their high protein content and the presence of connective tissues which are digested more readily in an acid than in an alkaline medium the rôle of the stomach in the digestion of meats is one of greater importance than with certain other foods. For this reason it has been considered worth while to carry out a series of studies on the response of the stomach to meats. The beef products are among the most widely used of meats.

The digestion time of meats in the human stomach has been studied by Beaumont (1), Jessen (2) and Penzoldt (3). The values commonly quoted in the literature are the results of their investigation. Beaumont's experiments were carried out on a single man; the hunter, Alexis St. Martin, who possessed a permanent gastric fistula. Beaumont records some experiments on beef and beef products, in most cases the meat fed being but a part of the mixed meal which also included water *ad libitum*. The amounts of meat fed were ordinarily regulated only by the appetite of the subject. Beaumont's results were for a long time the only ones available. His findings on beef are shown in table 1.

Jessen's subject was a man thirty years old. He was given 100 grams of meats of various kinds with 300 cc. of water. The stomach was emptied after a couple of hours. If meat fibers remained, the test was repeated on another day using a longer interval. The data given in table 2 were obtained as the result of five tests.

[1] The expenses of this investigation were defrayed by funds furnished by Mrs. M. H. Henderson, the Curtis Publishing Company and Dr. L. M. Halsey.

Penzoldt used two men as subjects, one being given 250 grams of meat, the other 100 gram portions. Water ingestion was not limited, as much as 1200 cc. being given in some cases. Toward the estimated end of digestion, samples were pumped out every 15 minutes, using a large stomach tube, until the stomach had been emptied. A little

TABLE 1

Digestion time of beef in the human stomach (Beaumont)

KIND OF MEAT	COOKING	HOURS	MINUTES
Beef	Roast rare	3:00	
Beef	Roast well-done	3:00	30
Beef steak	Broiled	3:00	
Beef boiled	Boiled	2:00	45
Beef liver	Broiled	2:00	

TABLE 2

Digestion time of beef in the human stomach (Jessen)

KIND OF MEAT	COOKING	EVACUATION TIME
		hours
Beef	Raw	2
Beef	Boiled medium	2½
Beef	Boiled well-done	3
Beef	Roast medium	3
Beef	Roast well-done	4

TABLE 3

KIND OF MEAT	COOKING	PRAEGER 100 GRAMS	GIGGLBERGER 250 GRAMS
		hours	*hours and minutes*
Beef sausage	Raw	2¾	3:25
Beef steak	Fried warm	3½	4:15
Beef steak	Fried cold	3½	
Beef steak	Raw chopped	3½	3:10

meat and water was given after each sample to replace that removed. The results this author obtained are presented in table 3.

The present series of tests on beef were carried out on normal medical students. Over seventy complete experiments were made on twenty-five different subjects. The results of sixty-four of these experiments

are reported in the present paper. Most of these tests were started at 1:00 p.m., the residuum from breakfast being previously removed with the Rehfuss stomach tube. One hundred gram portions of the meats prepared in various ways were ingested, the stomach tube being usually kept in place, though in a few cases it was withdrawn during the period of mastication. The presence of the tube did not, in most cases, interfere with the chewing of the food. The subject was told to follow his regular habit as to mastication. From 15 to 25 minutes were ordinarily required. Samples of from 5 to 8 cc. volumes were withdrawn at 15 minute intervals until the stomach was empty. Determinations of free and total acid, pepsin and amino nitrogen were carried out according to the procedure previously described (4), the method for amino nitrogen being a formol titration method. Specimens were also examined as to amount and character of meat present, character of liquid and other features. The emptying time was shown by the disappearance of meat residues from the samples or by failure to aspirate anything from the stomach with the subject placed successively in four positions, on right side, left side, face downward and on the back. The emptying time was confirmed by giving at once 100 cc. of water as lavage and immediately withdrawing same. This lavage in all cases showed a very low acidity (from 1–10), was clear or slightly cloudy and contained no meat residues except occasionally traces adhering to the mucus present. The lavage water was recovered quantitatively within a few cubic centimeters in these cases.

Subjects were chosen in such a way that direct comparisons of the different methods of cooking, etc., could be made under similar conditions. The subjects were at rest, that is, reading magazines, playing cards, copying notes or studying.

Table 4 gives a summary of the results obtained with beef prepared in different ways. The subjects are classified as having rapid- or slow-emptying stomachs. This classification has been found necessary because of the fact that there are marked differences between the emptying times of the stomachs of different entirely normal individuals under exactly the same conditions, this rapid or slow emptying being characteristic of a given stomach and very constant. The classification into types as used in this table is based not only on experiments with beef but on numerous experiments with other foods. About half of the men observed fall into each class. Nothing but confusion can result from a disregard of the marked differences in the response of the stomachs of different entirely normal individuals to the same stimuli.

TABLE 4

Response of the human stomach to beef and beef products

NO.	SUBJECT	FOOD AND PREPARATION	TYPE OF STOMACH							
			Rapid-emptying type				Slow-emptying type			
			Evacuation time, hours and minutes		Highest total acidity*		Evacuation time, hours and minutes		Highest total acidity*	
				average		average		average		average
1	Tri	Roast beef rare	2:45		70					
2	Ri	Roast beef rare	2:00		110					
3	Joa	Roast beef rare	2:45		90					
4	Cru	Roast beef rare	2:30		130					
5	Gl	Roast beef rare	2:45	2:30	110	102				
6	Sim	Roast beef rare					3:30		120	
7	Re	Roast beef rare					2:45		130	
8	Wal	Roast beef rare					2:30		130	
9	Oa	Roast beef rare					3:00	3:00	112	120
10	Ara	Roast beef medium	1:45		122					
11	Bos	Roast beef medium	2:45	2:15	122	122				
12	Sim	Roast beef medium					3:15		120	
13	Ral	Roast beef medium					3:45		108	
14	Kon	Roast beef medium					3:15	3:30	110	113
15	Ri	Roast beef well-done	2:00		105					
16	Lun	Roast beef well-done	2:30	2:15	123	114				
17	Ca	Roast beef well-done					3:30		131	
18	Con	Roast beef well-done					3:45		126	
19	Joe	Roast beef well-done (250 grams)					3:15		128	
20	Ne	Roast beef well-done (250 grams)					3:45	3:30	164	138
21	Con	Roast beef well-done (250 grams)					5:45	5:45	138	138
22	Hou	Steak hamburger round raw					3:00		110	
23	Sim	Steak hamburger round raw					3:00	3:00	113	111
24	Mor	Steak hamburger round (well-done)	2:00	2:00	98	98				
25	Kon	Steak hamburger round (well-done)					3:00		118	
26	Lec	Steak hamburger round well-done					3:00	3:00	120	119
27	Ri	Steak hamburger chuck well-done	2:00	2:00	109	109				
28	Joa	Steak rump tough rare	2:00		95					
29	Tri	Steak rump tough rare	2:30	2:15	135	115				
30	Joa	Steak rump well-done	2:00		93					
31	Tri	Steak rump well-done	2:45	2:30	153	123				
32	Swe	Steak shank tough well-done	2:30	2:30	111	111				
33	Con	Steak shank tough well-done					3:45	3:45	126	126

TABLE 4—*Concluded*

NO.	SUBJECT	FOOD AND PREPARATION	TYPE OF STOMACH							
			Rapid-emptying type				Slow-emptying type			
			Evacuation time, hours and minutes		Highest total acidity*		Evacuation time, hours and minutes		Highest total acidity*	
				average		average		average		average
34	Ara	Steak sirloin medium	3:00	3:00	128	128				
35	Kna	Steak sirloin medium					3:45	3:45	135	135
36	Joa	Steak sirloin well-done	3:15		118					
37	Kli	Steak sirloin well-done	3:00	3:10	131	125				
38	Joa	Steak tenderloin medium	3:30	3:30	98	98				
39	Tri	Steak sirloin well-done	3:30	3:30	168	168				
40	Oa	Steak sirloin planked medium					3:00		151	
41	Mab	Steak sirloin planked medium					3:00	3:00	144	147
42	Cla	Steak sirloin planked well-done					3:15	3:15	79	79
43	Joa	Steak tenderloin planked medium	3:00	3:00	92	92				
44	Wal	Steak round boiled					3:45	3:45	139	139
45	Ne	Stewed beef					3:15		88	
46	Ral	Stewed beef					4:15	3:45	107	100
47	Ral	Stewed beef (250 grams)					6:00		147	
48	Ne	Stewed beef (250 grams)					5:30	5:45	117	132
49	Ara	Dried beef	2:15	2:15	120	120				
50	Bec	Dried beef					3:45	3:45	90	90
51	Bec	Beef tongue boiled					4:30		159	
52	Kar	Beef tongue boiled					4:00	4:15	131	145
53	Lun	Corned beef boiled	2:45	2:45	77	77				
54	Sim	Corned beef boiled					3:30	3:30	125	
55	Wal	Corned beef boiled					3:45	3:30	125	125
56	Cru	Bologna	2:45		130					
57	Bos	Bologna	2:30	2:45	105	118				
58	Ara	Liver, calves, fried	2:30		110					
59	Bos	Liver, calves, fried	3:15	2:45	135	122				
60	Gl	Frankfurters (80 grams)	2:15		118					
61	Ca	Frankfurters	2:45	2:30	109	114				
62	Spk	Sweetbreads	2:15	2:15	85	85				
63	Tri	Tripe	3:30	3:30	125	125				
64	Oa	Tripe					3:15	3:15	110	110

* Acidity expressed in cc. of N/10 alkali required to neutralize 100 cc.

This was clearly brought out by a comparison of the average evacuation times of the two types of subjects on beef. These were found to be 2 hours 35 minutes for subjects of the rapid type and 3 hours 25 minutes for subjects of the slow-emptying type. Note also the three tests on subject "Ri" (expers. 2, 15 and 27) with roast beef and beef steak, the evacuation time in each instance being 2 hours, acidities varying only from 105–110. Compare this with tests on one of the men of a slow-emptying type, e.g., subject "Con" who showed evacuation times of 3 hours 45 minutes each on roast beef and beef steak (expers. 18 and 33) and gave in each case an acidity of 126. The response of a normal subject as regards the forms of the acid curves and the evacuation times are constant within quite narrow limits when tests are carried out on different days using similar foods. The acidities vary somewhat more than the emptying times. It is of course possible in spite of the large number of subjects used and tests made and cross checking wherever practicable, that in some cases the results are influenced to a slight extent by uncontrolled variations in individual response, which in general show a remarkable constancy. Very seldom was an instance noted where a stomach of the slow type emptied sooner than one of the rapid type on a given food. Experiments 63 and 64 on tripe show such a condition and must be explained on the basis of a personal idiosyncrasy of one of the subjects for this particular food.

It is necessary to carefully distinguish rapid emptying with high acidity (a normal response), from rapid emptying with low or anacidity which is usually pathological, and is readily brought about, for example, by a cold. No experiments on the latter class of individuals are reported here.

The curve of acidity may be taken as a measure of a glandular response of the stomach. With meats this response gave the highest normal total acid (184 cc. N/10 KOH − 100 cc. gastric juice) that has been obtained on any class of food in this laboratory. The acidity due to free HCl in this case was 133. Carlson found that pure gastric juice from his one subject, Mr. V., did not rise above an acidity of 0.48 per cent HCl, which is equivalent to 132 cc. N/10 KOH required to neutralize 100 cc. of gastric juice. The average high point of the acid curves in our beef tests was 120 with many instances of acidities between 130 and 160. These acidities are far higher than the values usually accepted by physiologists and clinicians for the acidity of human gastric juice and gastric contents. Values which have been held to represent conditions of marked pathological hyperacidity are found to be within the average

range of normal subjects and require a revision of our ideas as to what constitutes "hyperacidity."

The total acid is the strength of the gastric juice as secreted minus the lowering produced by the neutralizing and diluting effect of ingested food and saliva and possible regurgitated duodenal contents. After taking the test meal all saliva was expectorated into a beaker furnished the subject and kept constantly by him, so that after eating the error introduced by the esophagus was probably small. The diluting effect produced by ingested material therefore grows smaller and smaller as digestion proceeds and the gastric juice continues to be poured out in increasing ratio to the original volume. In the meat tests especially the primary amount is comparatively small when the total volume of juice is considered. This has been estimated by Carlson (5) to be as much as 700 cc. for a single meal.

The neutralization effected by regurgitation of duodenal contents is inconstant, but fortunately when digestion is going on with chyme passing into the duodenum there is practically a constant flow also of pancreatic juice into the duodenum, so that regurgitation at this time is evidenced by trypsin in the stomach. The presence of bile with regurgitation is variable, with foods which do not stimulate the production of bile. Meats, however, do cause a flow of bile so that in most cases in which there was found a significant drop of the acid before the final downward curve, bile could be demonstrated in the sample.

No association could be found between visible evidence of regurgitation and the height of acidity in the stomach. There seemed to be a tendency for bile to appear oftener with the long-continued digestions. With this there was associated the factor of individuality in that some subjects constantly showed regurgitation at some point of the test meal, while others rarely did.

The total acid curves shown have, then, been influenced almost constantly by the amount of food ingested. The type of curve was further influenced by the constant flow of gastric juice and by inconstant regurgitation of duodenal contents which could be demonstrated by the presence of bile. The degree of total acidity given by the added gastric juice is the best measure of the response of the gastric glands.

The other chief factor in determining the curve given is the rate of passage of the food from the stomach. It is generally considered that this begins when the food at the pyloric end of the stomach has been prepared for the entrance into the duodenum. The rate of its passage is fixed by the rapidity of digestion in the stomach which in turn is deter-

mined by the activity of the stomach and the digestibility of the food. With a given acidity of the stomach, then, the digestibility of a food is indicated by the emptying time of the stomach. This relates principally to meats and other high protein foods which are subjected to considerable digestion in the stomach.

The motor function of the stomach which is a most important one is determined in a healthy man largely by individual characters. So with a given kind of food as a stimulus there are shown quick-emptying and slow-emptying stomachs. This time division is not absolute with different kinds of foods but is only relative to the types of stomachs.

Other analyses made upon the gastric juice were free acid, pepsin and the formol titration.

The free acid found in any fresh gastric sample may be influenced by the total acidity, the rapidity of secretion of the gastric HCl and the rate of combination of the acid with food materials in the stomach. In general the free acid follows fairly well the trend of the total acid curve. The difference between the total acid and free acid which represents mainly combined acids might be taken to indicate the rate of protein digestion in the stomach.

The formol titration of Sörenson has been performed with the hope that it might lead to valuable data upon the rate of protein cleavage in the stomach. This was used by Zunz (6) in working upon the human stomach contents and the stomach contents of cats and dogs. He found no definite relationship between the time the food was eaten, the degree of hydrolysis of the food, the amounts of ammonia or amino nitrogen split off and the free or total acid secreted.

Christiansen (7), however, working with human gastric contents found that the increase in bound (combined) HCl is equivalent to that of the free amino groups determined.

The formol titration includes not only the free amino groups of the gastric contents but also the ammonia of the gastric juice and of the meats fed, and a slight amount from the saliva. Carlson (8) reports that his gastric fistula subject showed an average of 3 mgm. ammonia nitrogen in 100 cc. of gastric juice. The amount of ammonia nitrogen in fresh raw beef ranges from 3 to 10 mgm. in 100 grams of meat (9), (10).

In general the curves show a tendency to rather high initial values of formol-titratable nitrogen. They then tend to follow the total acid curve upward, but break downward somewhat more quickly than the final drop of the total acid curve, and generally end low as compared with the initial point.

The high beginning is probably due to the rapid extraction of ammonia nitrogen from the finely divided meat ingested. The rise in the curve thereafter is due then to the splitting of proteins, with the liberation of more amino groups and slight amounts of ammonia. Since, however, most of the protein products are not final products the amount of amino groups formed is comparatively small, so that a slight rise in the amino curve probably indicates considerable protein cleavage. This rise of the curve is undoubtedly minimized also by the fact that the soluble products of digestion leave the stomach fairly rapidly. This last fact is also the explanation of the early downward break of the amino curve.

Pepsin was determined in a number of tests, but was not continued throughout the series. The charts show constantly that there was a rise of the pepsin curve which became more marked toward the end of digestion. Furthermore, consecutive samples may show pepsin values which

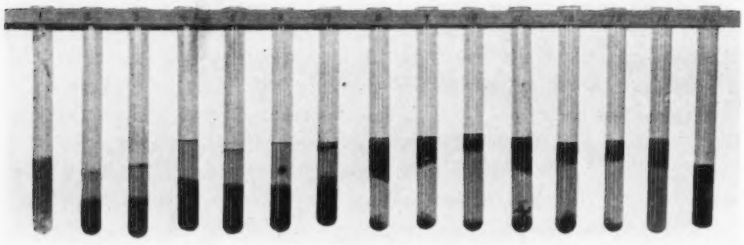


Fig. A1

vary widely out of the general course of the curve, so that no reliance can be placed in any sample as showing the true peptic strength of the gastric juice as secreted. The reason for this is found in the meat proteins which are so abundant and which adsorb pepsin easily, so that the juice as it is filtered leaves behind with the meat a large part of its pepsin content. As the meat is digested and leaves the stomach, the pepsin curve gradually rises due to the larger amount of that enzyme in proportion to the undigested proteins. A true test of the pepsin-secreting power of the stomach should be made with some stimulant which does not adsorb pepsin. Working with water and glucose solution, Fowler (11) showed in this laboratory that the pepsin content of the gastric juice is proportional to the height of total acidity up to a certain point. This would probably be found true also with meats were the difficulties of estimating the true value of pepsin to be overcome.

Passage of different constituents of meat from stomach. The meat which is taken as test meal is not all uniformly mixed, and disintegrated in the stomach, and passed into the duodenum as a homogeneous mass. As the characters of the samples were observed it was early noted that there is a separation of different parts of the meat which leave the stomach separately. The finely divided portions which settle to the bottom of a sample tube empty first, then the coarse fibrous material which is on top of the sample after standing, and finally the fatty material. The effect of gravity seems to play a considerable part in the emptying of the various constituents. This is illustrated by the photograph (fig. A1) and following description of samples obtained after a test meal of meat had been given to a normal subject.

Description of samples

1. 11 cc. Layer fat at top 2 mm. fine globules. Solids 0.7. White. Fluffy. Fibrous. Fibers held in bunches in mucus. Shows globules (fine) of fat in fibers. Liquid—water clear.

2. 9 cc. Fat up 2 mm. fine globules. Solids 0.5 down, very fine fibers which are separated. Mucus of previous sample all dissolved. Very fine fat globules mixed in. Light brownish pink. Liquid—very slightly turbid. Whitish.

3. 10 cc. Trace of fat up. Solids 0.5 down. Like no. 2 except no fat mixed in.

4. 13 cc. Solids 0.5 down. Like no. 2. Liquid—slightly turbid.

5. 12 cc. Like no. 4. Solids 0.5 down.

6. 13 cc. Solids 0.4 down. Material is more pinkish. Less of brown. More fluffy.

7. 12 cc. Solids 0.3 down and 0.1 up. Light brown.

8. 13 cc. Trace of solids down and 0.4 up and 2 mm. solids fat up. Material up is coarse fibers clumped in masses and light brown.

9. 12 cc. Solids 0.1 down and 0.3 up. Trace fat. Same as no. 8. Down more brownish. Liquid—clear.

10. 13 cc. Solids 0.1 down and 0.2 up. Like before. Sediment is fine yellowish. Liquid—turbid. Very light yellow (slight regurgitation).

11. 13 cc. Solids 0.3 up. Same as sediment of no. 10. 0.1 down. Trace of fat up.

12. 14 cc. Solids 0.3 up and 0.1 down. Trace fat up.

13. 13 cc. Solids 0.2 up ($\frac{1}{2}$ coarse fibers and $\frac{1}{2}$ fat). Trace down of fine yellow. Liquid—very slightly turbid. Very slight trace yellow.

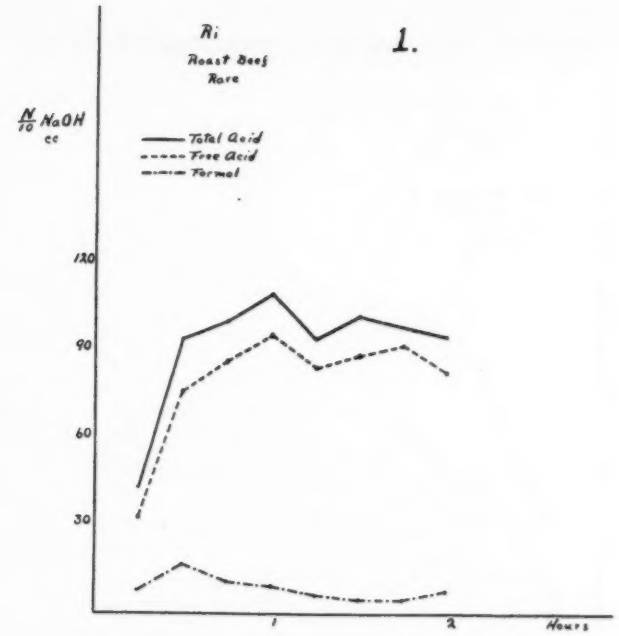
14. 14 cc. Solids 0.3 up ($\frac{1}{2}$ coarse fibers and $\frac{1}{2}$ fat). Trace down fine yellow. Liquid—very slightly turbid. Very slight trace yellow.

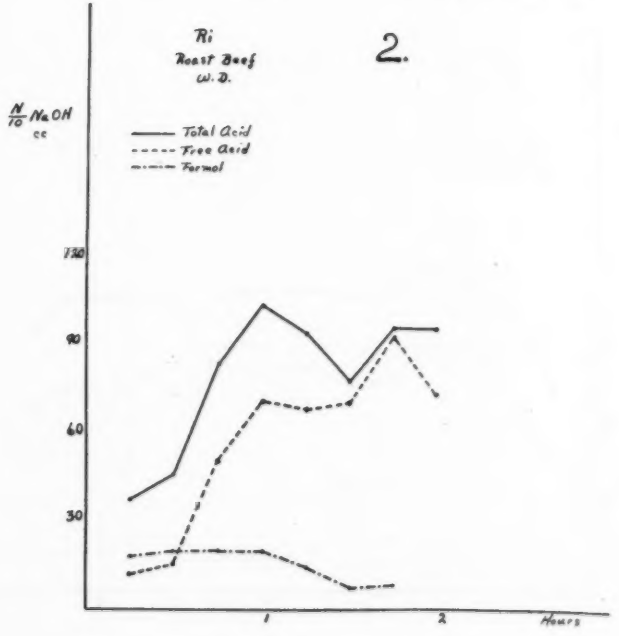
15. 9 cc. Considerable (about 0.2) very fine light brown granular material throughout the solution. Trace fat up. Probably washed from walls of stomach.

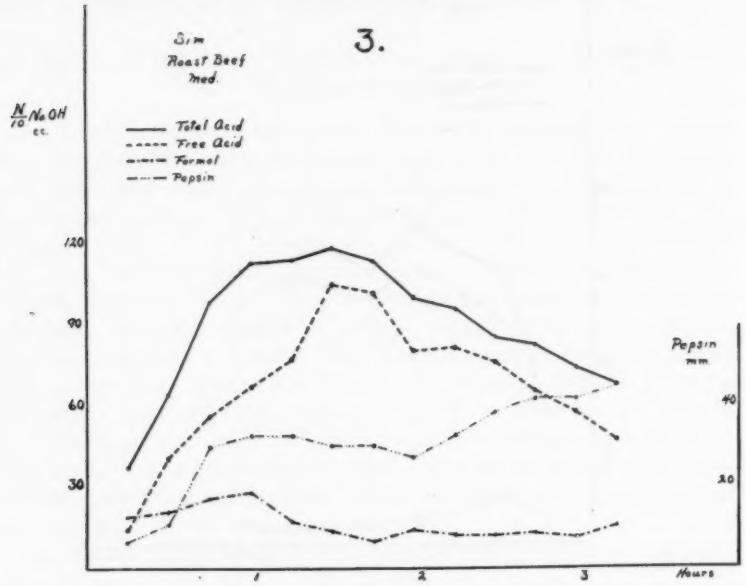
DISCUSSION OF RESULTS OBTAINED WITH DIFFERENT FORMS OF BEEF AND BEEF PRODUCTS

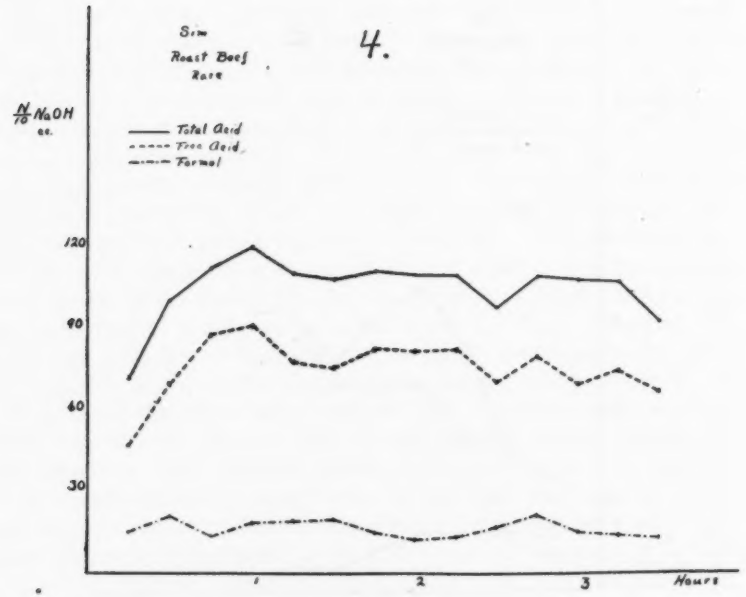
Roast beef. Figures 1 and 2 show the results obtained after feeding rare and well-done roast beef respectively to a man of the rapid-emptying type. Figures 3 and 4 show the comparative effects produced by rare and medium well-done roast beef in an individual of the slow-emptying type. It will be noticed that as far as maximum acidities developed and evacuation time were concerned the mode of cooking showed very little effect. The averages given in table 4 show the same tendency. The rare roast beef may be slightly more easily digested than the medium or well-done. Our figures for rare and well-done roast beef on the slower type of stomachs are the same as those obtained by Beaumont; 3 and $3\frac{1}{3}$ hours respectively. In no case however was a variation as great as that of Jessen found who noted in his subject an evacuation time of 3 hours for medium and 4 hours for well-done beef. Grindley found that different methods of cooking meat had no influence upon utilization in the human organism (12).

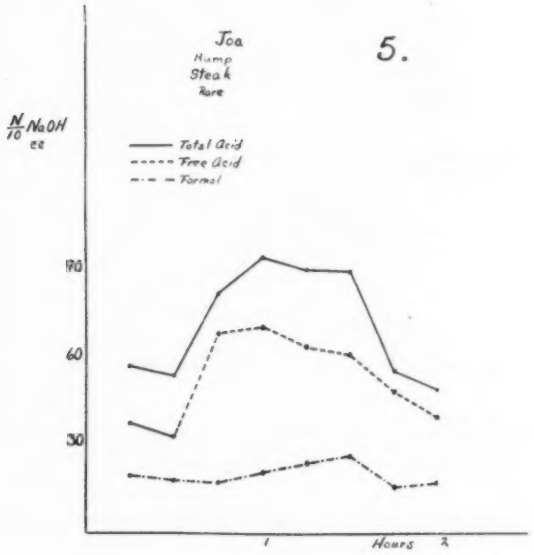
Beef steaks. Steaks from several different cuts of beef prepared in different ways were tested. For average results see table 4. The evacuation times for men of the rapid type varied from 2 to $3\frac{1}{2}$ hours and for the slow men 3 to $3\frac{3}{4}$ hours. Varying the degree of cooking seemed to have little effect. Neither rump, shank, sirloin nor tenderloin steaks showed more than 15 minutes difference in time of digestion of rare, well-done or medium-well-done products, with one exception which must be otherwise explained. Some differences were, however, noted with regard to the various cuts of beef used. The cheaper and tougher cuts of meat showed more rapid evacuation from the stomach than more expensive and more tender cuts such as tenderloin. This is brought out clearly by the summary of averages given in table 4. Curves showing the response of the same individual to rare and well-done steaks of the same cut (rump) are shown in figures 5 and 6. These should also be compared with figures 7, 8 and 9 showing the reactions of the same individual to a sirloin, a tenderloin and a planked tenderloin steak. It will be noted that these latter required from an hour to an hour and a half longer to leave the stomach and that planking reduced slightly the time required. The difference in favor of the cheaper and tougher cut is also clearly shown by a comparison of figures 10 and 11. The cheap cuts of meat used and the steaks prepared from them were extremely tough and were chosen particularly with a view to comparing the digestion of the toughest obtainable steak with the digestion of tenderloin

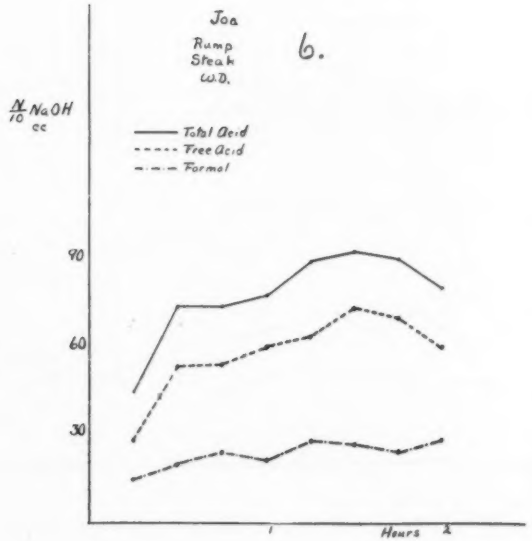

Ri
Roast Beef
Rare
1.
N/10 NaOH
cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
Hours

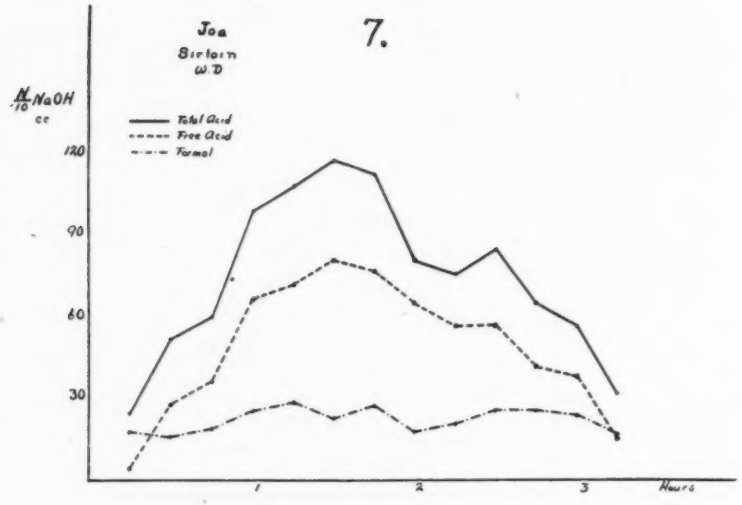

Ri
Roast Beef
W. D.
2.
N/10 NaOH
cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
Hours

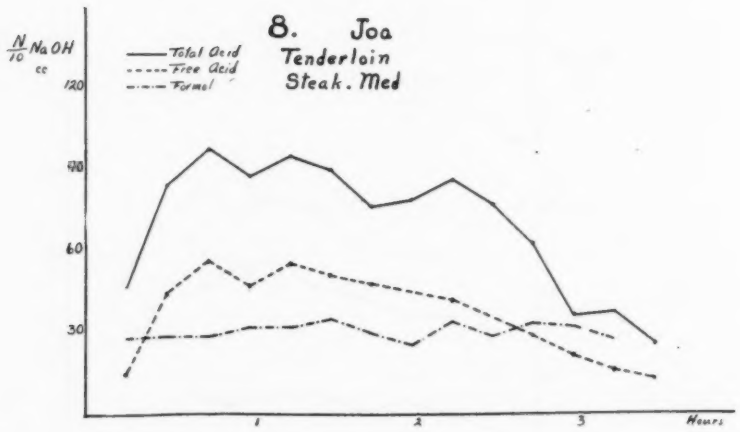

3.
Sim
Roast Beef
Med.
N/10 NaOH cc.
Total Acid
Free Acid
Formol
Pepsin
120
90
60
30
Pepsin mm.
40
20
1
2
3
Hours

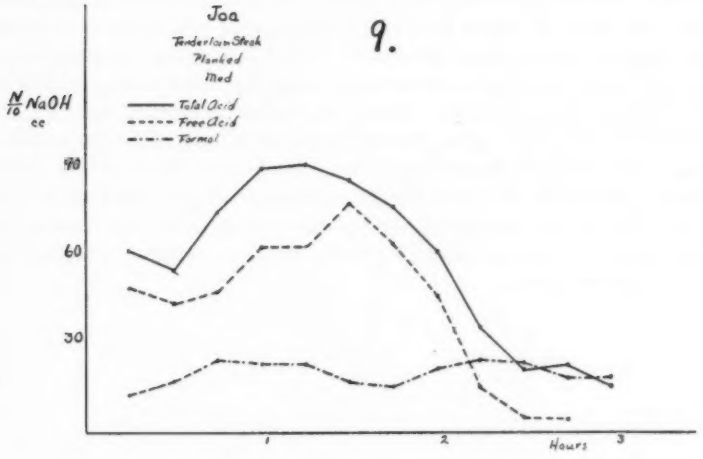

4.
Sim
Roast Beef
Rare
N/10 NaOH cc.
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours

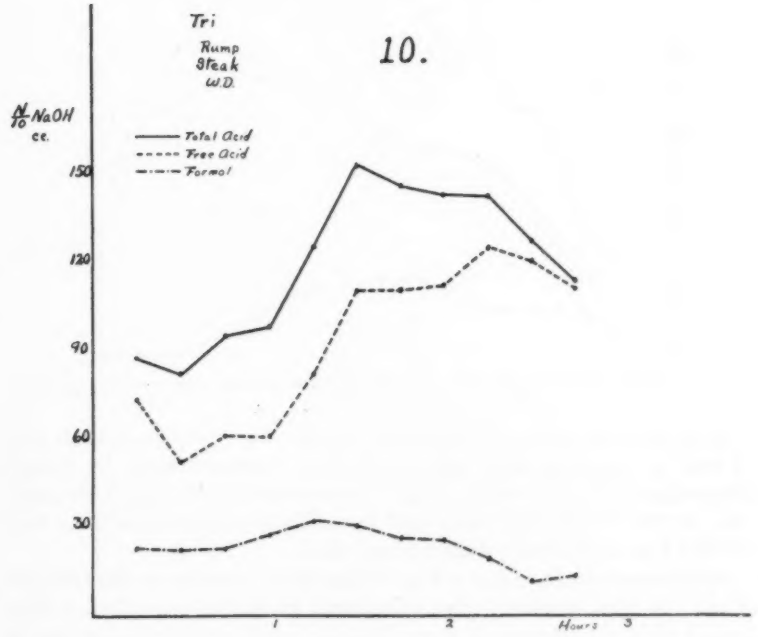

Joa
Rump
Steak
Rare
5.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
60
30
1
Hours
2


Joa
Rump
Steak
W.D.
6.
N/10 NaOH cc
Total Acid
Free Acid
Formol
90
60
30
1
Hours
2

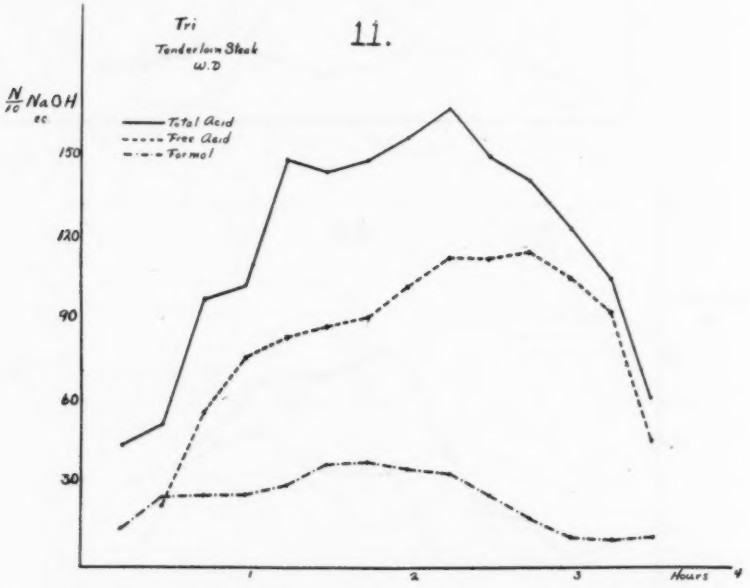

Joa
Sirloin
W.D
7.
N/10 NaOH
cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours


N/10 NaOH
cc
Total Acid
Free Acid
Formol
8. Joa
Tenderloin
Steak. Med
120
90
60
30
1
2
3
Hours


Joa
Tenderloin Steak
Planked
Med
9.
N/10 NaOH
cc
Total Acid
Free Acid
Formol
90
60
30
1
2
Hours
3

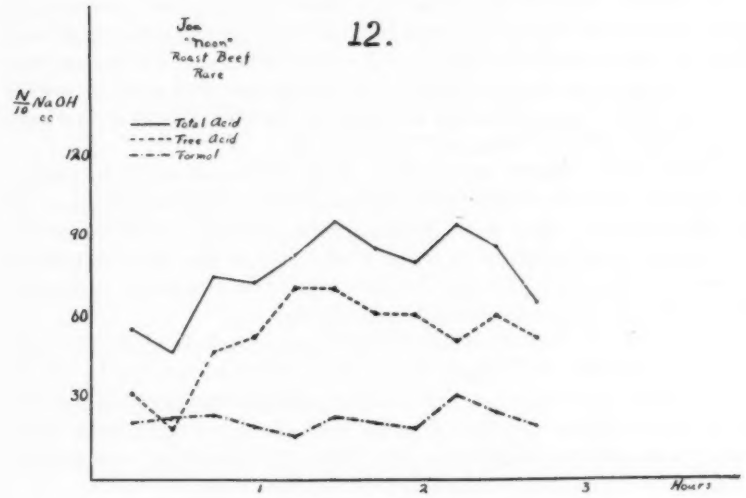

Tri
Rump
Steak
W.D.
10.
N/10 NaOH
cc.
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
Hours
3

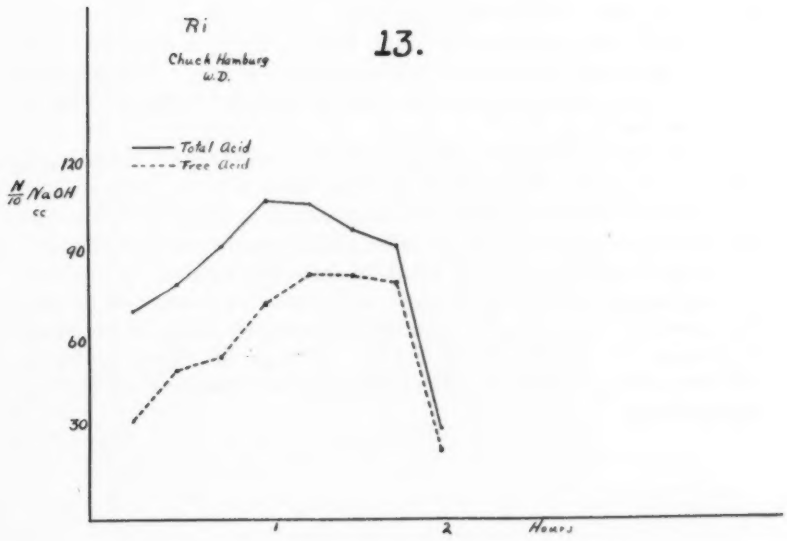
steaks. These latter were prepared by the chef of a high grade restaurant and were of course far more appetizing in appearance and taste as well as more tender than the others. That the cost of the different cuts of beef bears no relation to nutritive value has been shown particularly by Hall and Emmett (13). Cost is dependent upon such considerations as tenderness, grain, color, general appearance and convenience of cooking. According to the authors cited, the choice portion of beef forming about one-fourth its total weight represents nearly one-half its retail cost. From the standpoint of protein and energy, the cheaper cuts were found to be as valuable and in some cases more valuable than higher-priced cuts.



It is fortunate that our stomachs also give favor to the cheaper cuts of beef by digesting them more easily than expensive cuts. A typical comparison between roast beef and these steaks is obtained from figure 12. It will be seen that roast beef leaves the stomach sooner than tenderloin but more slowly than a rump steak.

Hamburger steaks. That a hamburger steak remains in the stomach about the same length of time as an equal amount of roast beef is illustrated by a comparison of these with the same individual (see figs. 3, 4 and 13). It may be that hamburger is handled a little more easily than roast beef but the difference is very slight.


Jon
"Noon"
Roast Beef
Rare
12.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours


Ri
Chuck Hamburg
w.D.
13.
Total Acid
Free Acid
N/10 NaOH cc
120
90
60
30
1
2
Hours

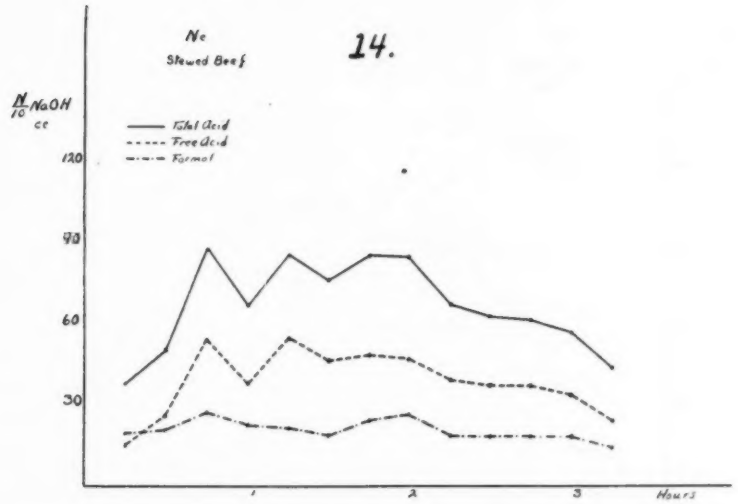
Stewed beef. Figures 14, 15, 16 and 17 show comparisons between roast beef and stewed beef on the same individuals. In one case the stewed beef left the stomach the sooner while in the other case the reverse was true. In one case the acid stimulation was greater with roast than with stewed beef. Apparently the response of the stomach to stewed beef is not much different from that to roast beef.

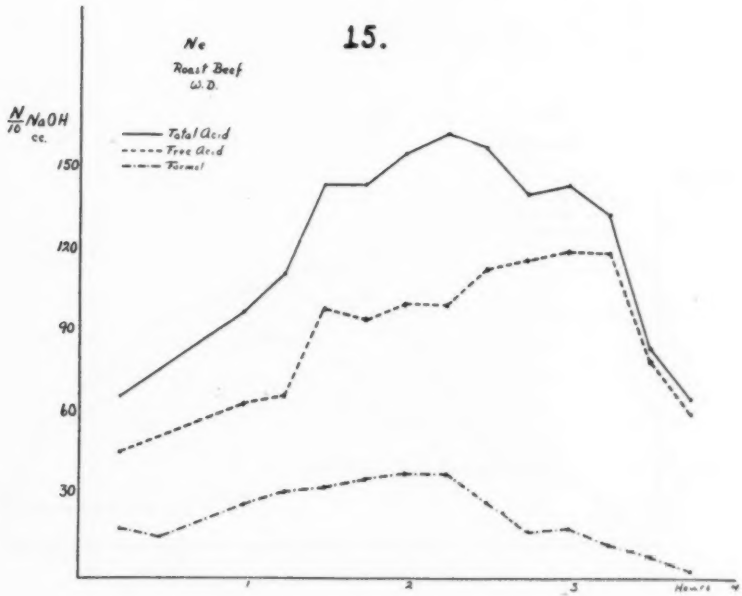
Corned beef. Boiled corned beef shows little difference in gastric response from that of roast beef or stewed beef. Evacuation times are about the same. The boiled meats appear, however, to be somewhat less active in stimulating secretion as the rise in the curve is usually somewhat delayed. This may be due to loss of extractives. Compare figure 18 with figures 3 and 4 and figures 19 and 20.

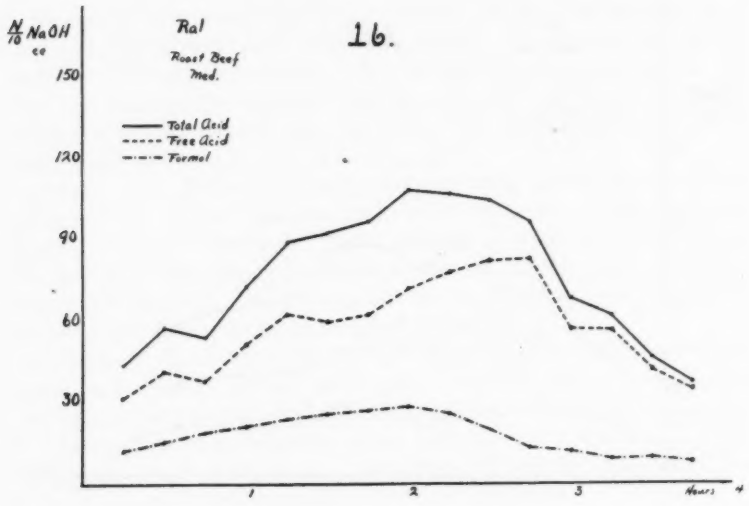
Dried beef. In the case of dried beef 70 grams were fed instead of 100 grams on account of the high solid content of this form of beef. Dried beef appears to be about as digestible as ordinary roast beef, but the lack of moisture where no water is taken with the meat appears to delay slightly the onset of digestion. See figures 21 and 22 for a comparison of dried and roast beef on a subject of the rapid type.

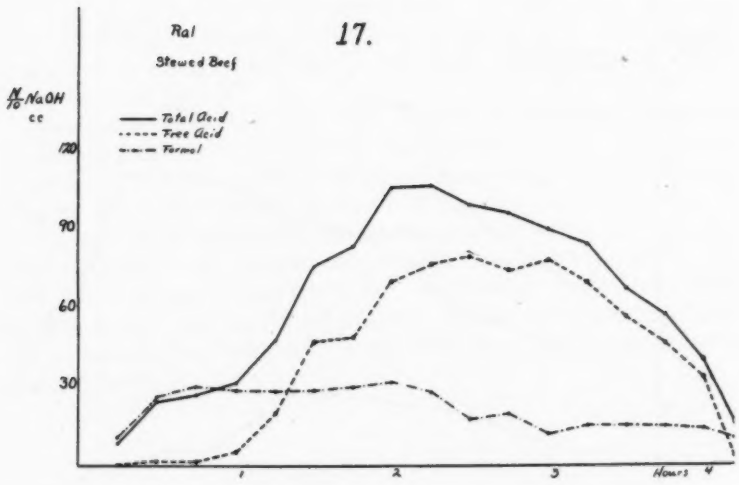
Beef bologna, calves' liver and beef tongue. Beef bologna was found in two cases to be digested in practically the same time as roast beef (see figs. 23 and 24). Calves' liver required a slightly longer time to leave the stomach than roast beef or dried beef. This is not in agreement with the finding of Beaumont (compare figs. 22 and 25). Boiled tongue was found in a single case to take over $4\frac{1}{4}$ hours as compared with $3\frac{3}{4}$ hours for dried beef.

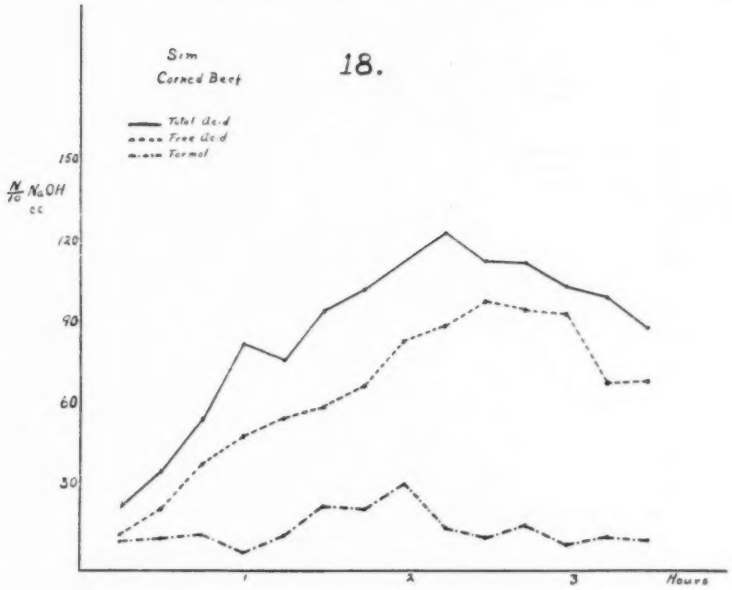
Frankfurters, sweetbreads and tripe. Beef frankfurters left the stomach rather more quickly than most of the other forms of beef. For example, 80 grams of boiled frankfurter left the stomach in $2\frac{1}{4}$ hours in one case (fig. 26) as compared with $2\frac{3}{4}$ hours for 100 grams of roast beef (fig. 27). Calf sweetbreads required $2\frac{1}{4}$ hours to leave the stomach in our test, thus belonging to the class of more rapidly moving meats (see fig. 28). The so-called "honeycomb" tripe left the stomach a little more slowly than roast beef (see figs. 29, 30 and 31). The gastric digestion of tripe did not appear, however, to differ materially from the digestion of other beef products.

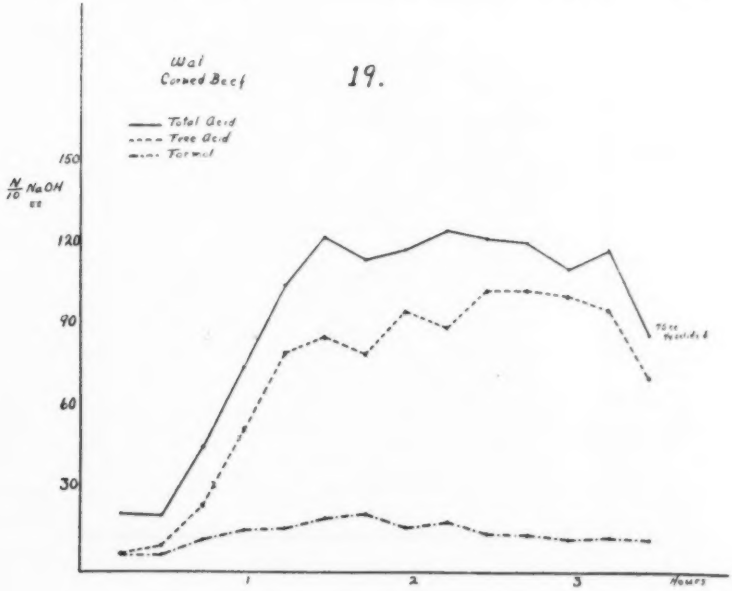

Ne
Stewed Beef
14.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours

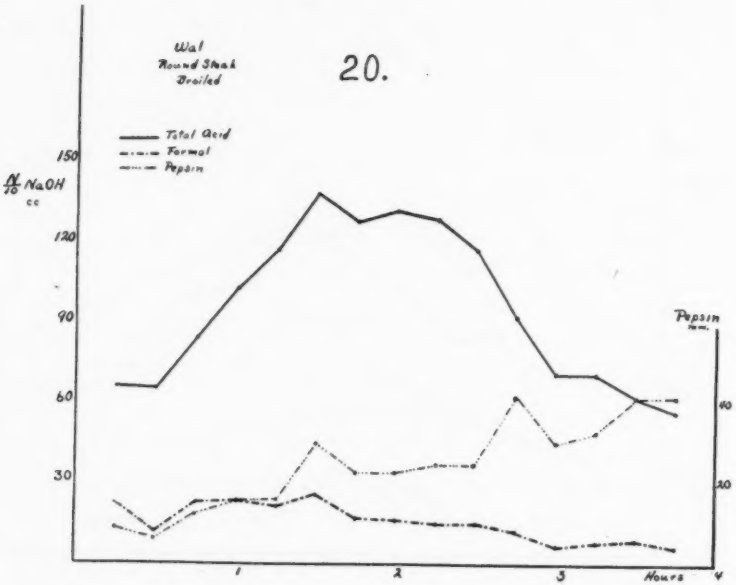

Ne
Roast Beef
W. D.
15.
N/10 NaOH cc.
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
Hours

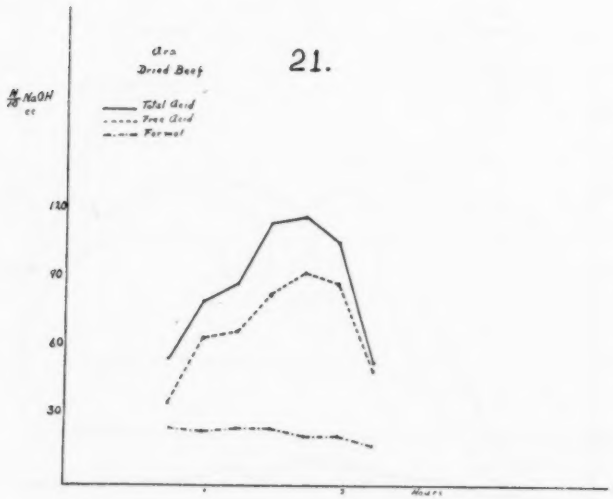

N/10 NaOH
cc
Ral
Roast Beef
Med.
16.
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
Hours
4

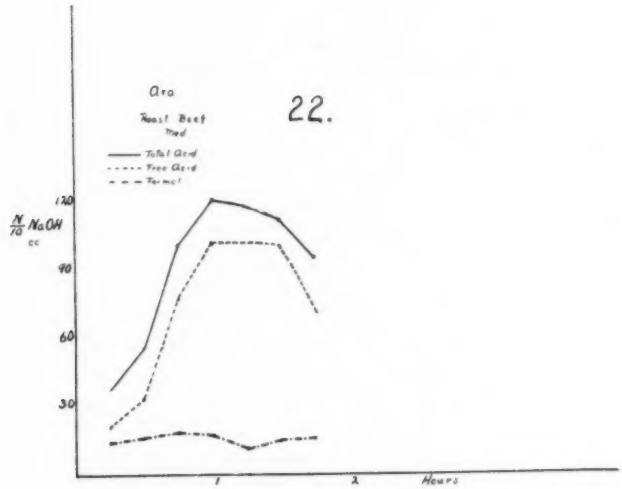

Ral
Stewed Beef
17.
N/10 NaOH
cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours
4

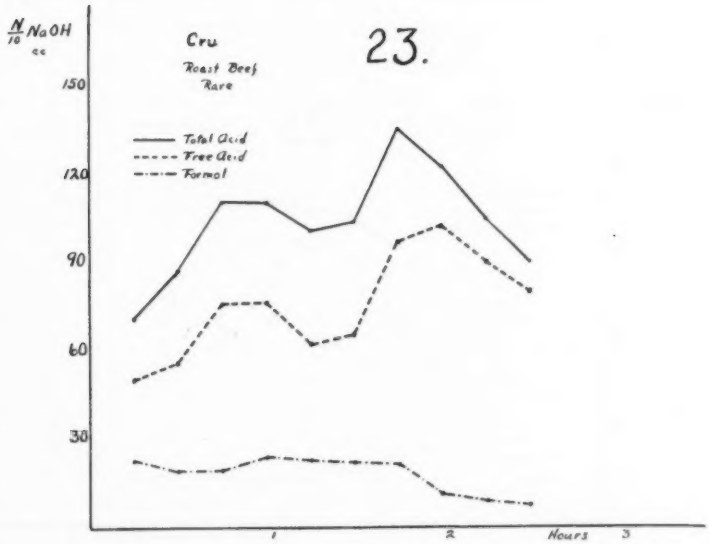

Sim
Corned Beef
18.
Total Acid
Free Acid
Formol
150
120
90
60
30
N/10 NaOH cc
1
2
3
Hours

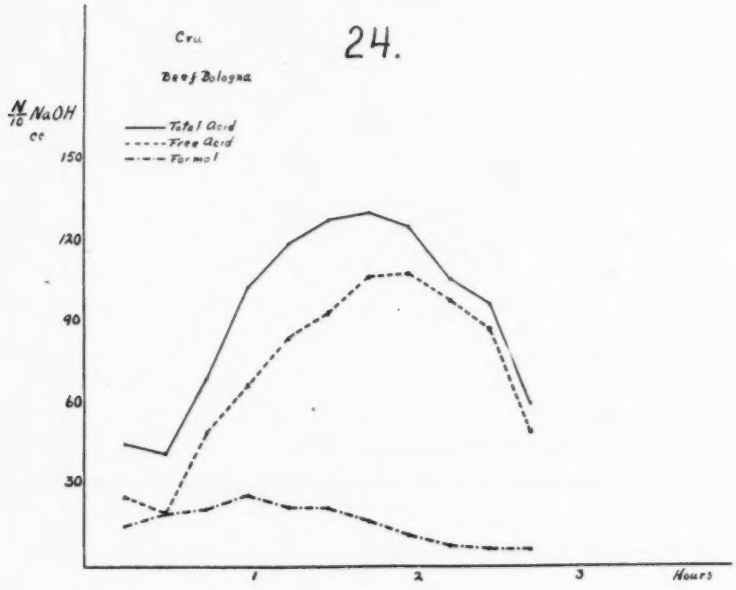

Wal
Corned Beef
19.
Total Acid
Free Acid
Formol
150
120
90
60
30
N/10 NaOH cc
1
2
3
Hours

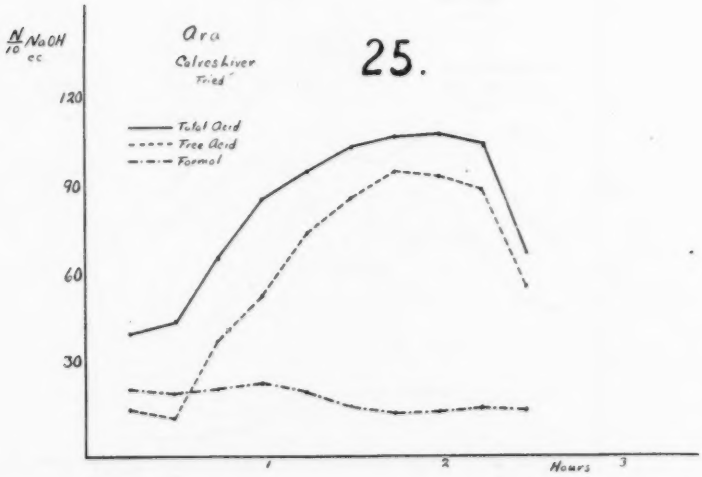

Wal
Round Steak
Broiled
20.
Total Acid
Formol
Pepsin
1/10 NaOH cc
150
120
90
60
30
Pepsin mm.
40
20
1
2
3
Hours
4

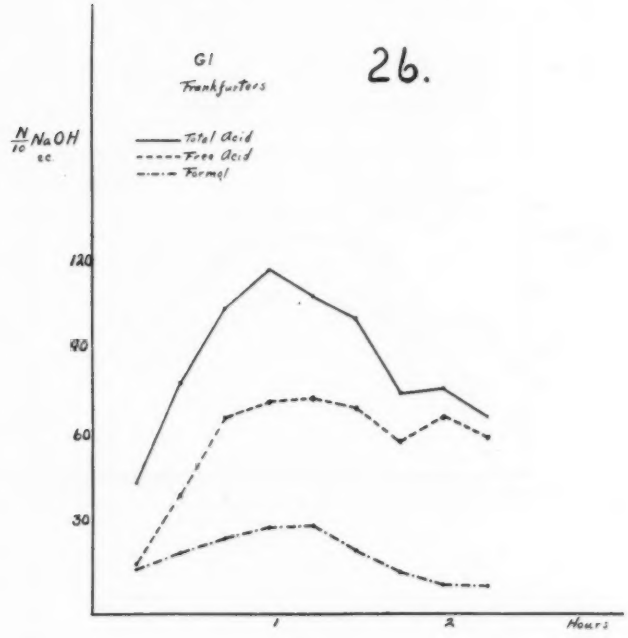

Ara
Dried Beef
21.
1/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
Hours

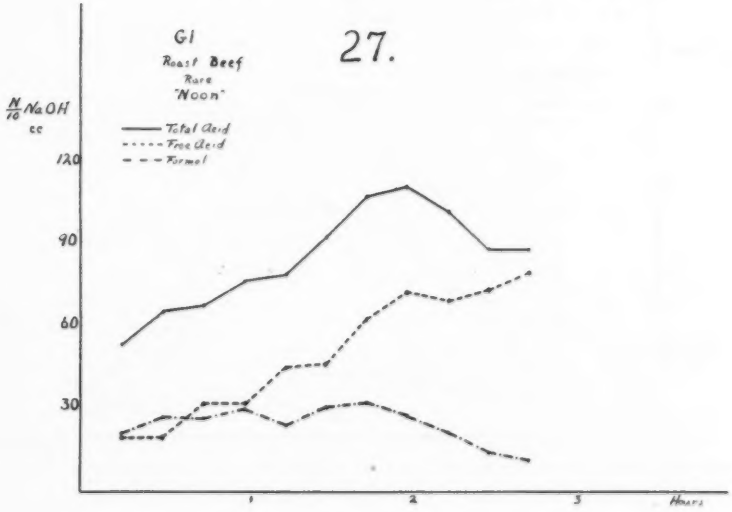

Ara
Roast Beef
Med
22.
Total Acid
Free Acid
Formol
120
90
60
30
N/10 NaOH
cc
1
2
Hours

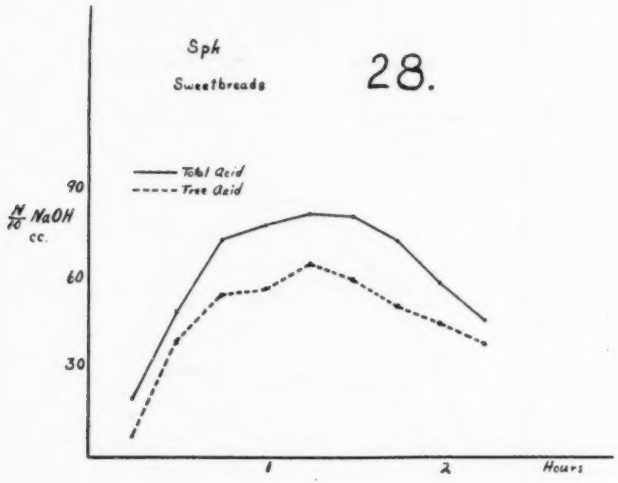

N/10 NaOH
cc
Cru
Roast Beef
Rare
23.
150
120
90
60
30
Total Acid
Free Acid
Formol
1
2
Hours
3

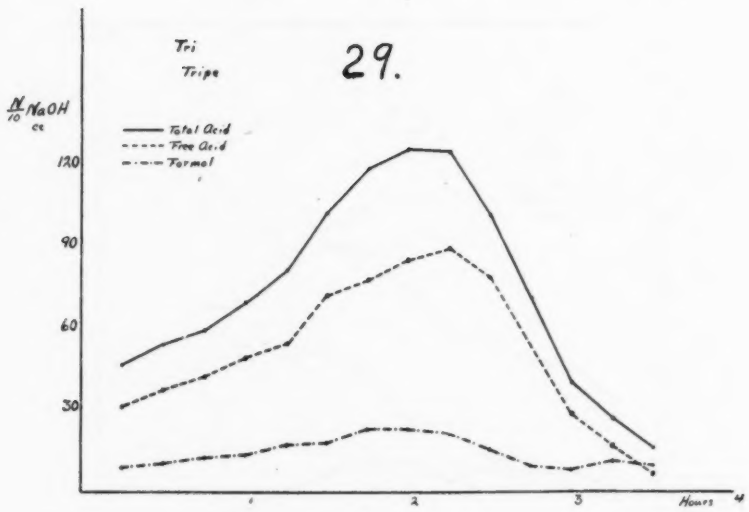

Cru
24.
Beef Bologna
N/10 NaOH cc
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
Hours

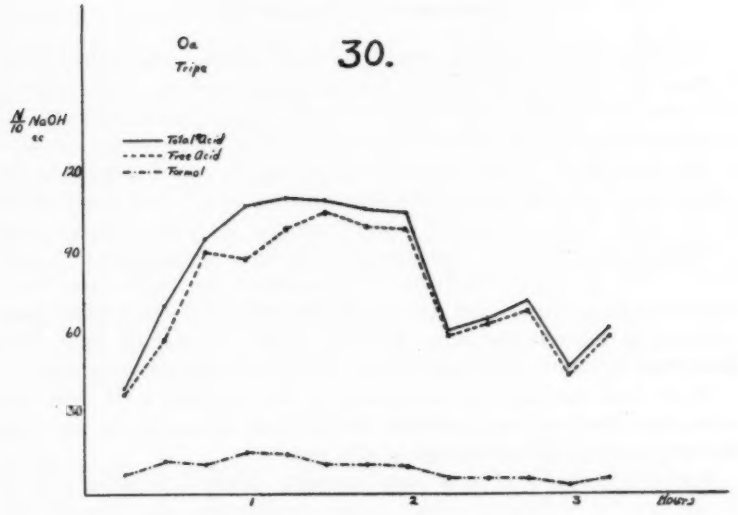

N/10 NaOH cc
Cru
Calves liver
Fried
25.
Total Acid
Free Acid
Formol
120
90
60
30
1
2
Hours
3

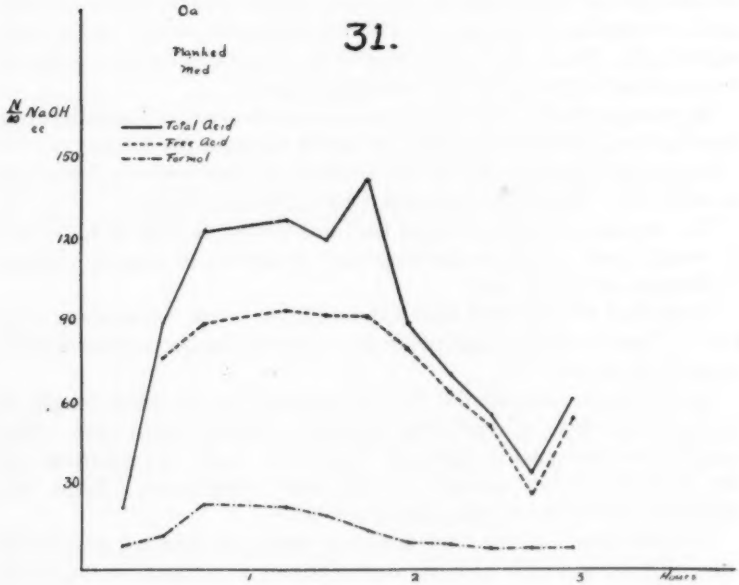

GI
Frankfurters
26.
N/10 NaOH cc.
Total Acid
Free Acid
Formol
120
90
60
30
1
2
Hours


GI
Roast Beef
Rare
"Noon"
27.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours


Sph
Sweetbreads
28.
Total Acid
Free Acid
N/10 NaOH cc.
90
60
30
1
2
Hours


Tri
Tripe
29.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours
4


Oa
Tripe
30.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours


Oa
Planked
Med
31.
N/10 NaOH cc
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
Hours

SUMMARY AND CONCLUSIONS

The digestion of various forms of beef and beef products in the normal human stomach was studied by the fractional method. Over seventy complete experiments were made on twenty-five different subjects. It was found that all normal stomachs do not respond alike to the entrance of the same food. Some respond very promptly and decidedly to the entrance of food, whereas others respond very slowly and indifferently to the same food. Also one type of normal stomach empties very quickly whereas a second type evacuates slowly under like dietary conditions. The average results for individuals of the rapid- and slow-emptying types have been tabulated (see table 4) and typical comparisons of different meats or different modes of cooking have been illustrated by curves showing acidities developed and emptying times.

Roast beef appeared to be handled by the stomach with about the same ease whether rare, medium or well-done, although the rare beef had perhaps a slight advantage in this respect.

Beef steaks appeared to be just as readily digested if cooked rare as if medium or well-done. Very tough steaks from the cheaper and tougher cuts of meats such as rump and shank showed distinctly more rapid evacuation than sirloin or the best tenderloin steaks, in the same individuals. Roast beef was found to lie between these two classes of steaks in gastric response and evacuation time.

Hamburger steaks were found to leave the stomach in about the same time as an equal weight of roast beef under the same conditions.

Stewed beef was handled by the stomach in practically the same time as roast beef, but with a less rapid development of acidity.

The response to boiled corned beef was similar to that of roast beef or stewed beef. Acid productions may, however, be slightly delayed in the case of corned beef.

Dried beef was digested with almost the same ease as ordinary roast beef. There may be a slight delay due to the low moisture content if the meat is eaten dry.

Beef bologna was handled by the stomach in the same length of time as roast beef. Calves' liver required a slightly longer time. Beef tongue was less readily digested than dried beef. Frankfurters left the stomach rather quickly, as did also sweetbreads. Tripe was digested a little less rapidly than roast beef.

For 100 grams of the beef products tested an average evacuation time of 2 hours and 35 minutes was obtained on subjects of the rapid-

emptying type and of 3 hours and 25 minutes on subjects of the slow-emptying type. Total acidities as high as 184 were obtained on meats. The average total acidity at the height of digestion was in the case of beef products 120. These high acid values regularly shown by normal men necessitate a revision of older ideas of "hyperacidity."

The amino acid nitrogen values (which include ammonia) are moderately high at the beginning of digestion due to the ammonia of the meats, show a secondary rise as digestion proceeds and fall to a low level at the end of digestion as the soluble products leave the stomach.

Pepsin values attain their highest point toward the end of digestion.

The authors wish to express their appreciation of the coöperation of the students of Jefferson Medical College, who kindly acted as subjects of these tests.

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GASTRIC RESPONSE TO FOODS[1]

IV. The Response of the Stomach to Pork and Pork Products

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This study on the digestion of pork and pork products is one of a series (1) of studies on the response of the human stomach to various foods. There are comparatively few investigations that bear directly on the gastric digestion of pork and its products.

Beaumont (2) gives the following results for the evacuation time of pork products, the tests being carried out on the subject, Alexis St. Martin. Roast beef values of the same investigator are given for comparison.

TABLE 1

Digestion time of meats in the human stomach (Beaumont)

MEAT	COOKING	HOURS	MINUTES
Pork	Roast	5	15
Sausage	Broiled	3	20
Pigs' feet	Soused	1	
Beef	Roast rare	3	
Beef	Roast well-done	3	30

Jessen (3) on his subject obtained a value of three hours for raw pork as compared with two hours for raw beef.

Penzoldt (4) obtained the following values for pork and beef steak on his two subjects.

[1] The expenses of this investigation were defrayed by a fund furnished by Mrs. M. H. Henderson, the Curtis Publishing Company and Dr. L. M. Halsey.

TABLE 2

Digestion time of meats in the human stomach (Penzoldt)

MEAT	COOKING	PRAEGER 100 GRAMS	GIGGLBERGER 160 GRAMS
		hours	*hours and minutes*
Ham	Boiled	3¼	3:20
Ham	Raw	3¾	3:46
Beef steak	Fried warm	3½	4:15 (250 gm.)

The present series of tests on the digestion of pork and pork products in the stomachs of normal men was carried out in the same manner as the tests on beef described in a preceding article (1).

Table 3 gives a summary of the results as to evacuation time and highest total acidities attained for a number of these meats, the results obtained with slow- and rapid-emptying types of stomachs being averaged separately. The results of 31 separate tests on 10 different subjects are recorded.

Figures 1 to 24 illustrate the digestive response of the stomach to the various pork products as compared with each other and with the response of certain types of beef. The comparative tests were made upon the same individuals under as nearly identical conditions as possible and the illustrations given are typical of the results obtained. One hundred grams of meat were fed unless otherwise stated.

Pork has usually been considered a rather indigestible kind of meat. In explanation of this it is said that the high proportion of fat between the meat fibers forms a coating which the gastric juice can penetrate only with difficulty. Hence convalescents, or people with digestive disturbances of any kind, are warned to leave all pork products out of their diet. In spite of this fact, however, and in spite of the fact that a great mass of people of the United States abstain from pork on principle, the per capita consumption of pork in this country is estimated at a little more than 88 pounds per year, nearly 10 pounds more per capita than beef, and almost as much as all the other forms of meat combined—including beef.

In reality the normal human stomach finds no extraordinary difficulty in digesting pork. It is true that, as shown in table 3, the emptying time of pork averages somewhat longer than that of other common meats, and this relative slowness of digestion applies also to pork products. If pork taken with a mixed meal, however, retards stomach digestion appreciably, then it may well be avoided by weak stomachs.

TABLE 3

Gastric digestion of pork and pork products

NO.	SUBJECT	MEAT	TYPE OF STOMACH							
			Rapid emptying				Slow emptying			
			Evacuation time, hours and minutes		Highest total acidity*		Evacuation time, hours and minutes		Highest total acidity*	
				average		*average*		*average*		*average*
1	Ara	Roast pork	3:30		108					
2	Cru	Roast pork	2:15		112					
3	Dal	Roast pork	2:00		102					
4	Ev	Roast pork	3:00	2:45	155	119				
5	Wat	Roast pork					3:45		118	
6	Wr	Roast pork					3:30		160	
7	Spn	Roast pork					3:45	3:45	120	130
8	Spn	Roast pork (250 grams)					5:30	5:30	145	145
9	Oa	Sausage	2:45	2:45	104	104				
10	Gl	Sausage					3:15		108	
11	Fal	Sausage					2:45	3:00	104	106
12	Joa	Ham, fried	3:00	3:00	65	65				
13	Wal	Ham, fried					4:15	4:15	150	150
14	Bos	Ham, boiled	3:15	3:15	137	137				
15	Kru	Ham, boiled					3:00	3:00	140	140
16	Km	Ham, minced	2:00	2:00	115	115				
17	Bos	Ham, minced					3:00	3:00	120	120
18	Mes	Ham, baked					4:15		118	
19	Ra	Ham, baked					3:45	4:00		118
20	Joa	Ham, bologna	3:15	3:15	68	68				
21	Wel	Bacon, fried					4:30	4:30	115	115
22	Gl	Pigs' feet, soused					3:45		120	
23	Ca	Pigs' feet, soused					2:30	3:15	135	125
24	Ara	Liver and bacon, fried	3:00		94					
25	Bos	Liver and bacon, fried	2:30	2:45	137	116				
26	Kru	Liver and bacon, fried					3:30	3:30	125	125
27	Wel	Ham sandwich, fried					2:45	2:45	101	101
28	Gl	Scrapple, fried					3:45		85	
29	Oa	Scrapple, fried					3:45	3:45	85	85
30	Gl	Pork chops					4:00		129	
31	Ca	Pork chops					3:45	4:00	144	134

*Acidities are expressed as cc. of N/10 alkali required to neutralize 100 cc.

Roast pork. The response of the stomach to 100 grams of roast pork is charted in figure 1. The evacuation time, as shown, was $3\frac{3}{4}$ hours.

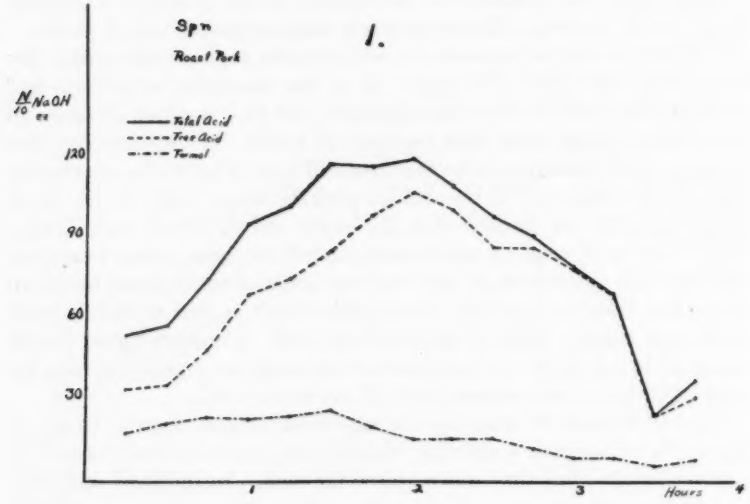
Figure 2 shows the response of another individual to roast pork. By comparing this chart with figure 22 in the preceding article on beef products (1) it will be seen that whereas roast beef required $1\frac{3}{4}$ hours to leave the stomach, roast pork required $3\frac{1}{2}$ hours. A comparison of the average results likewise shows for the rapid type of individual an emptying time for beef of $2\frac{1}{4}$ hours and for pork $2\frac{3}{4}$ hours, while for the slow-emptying type the periods were $3\frac{1}{2}$ hours and $3\frac{3}{4}$ hours respectively. Thus roast pork requires appreciably longer to digest than roast beef although the differences are not nearly so great as those found by Beaumont (see table 1). In fact certain individuals appear to handle roast pork very readily; fully as well as roast beef. Compare figure 3 with figure 23 in our paper on beef products showing an evacuation time for pork of $2\frac{1}{4}$ hours as compared with $2\frac{1}{2}$ hours for beef.

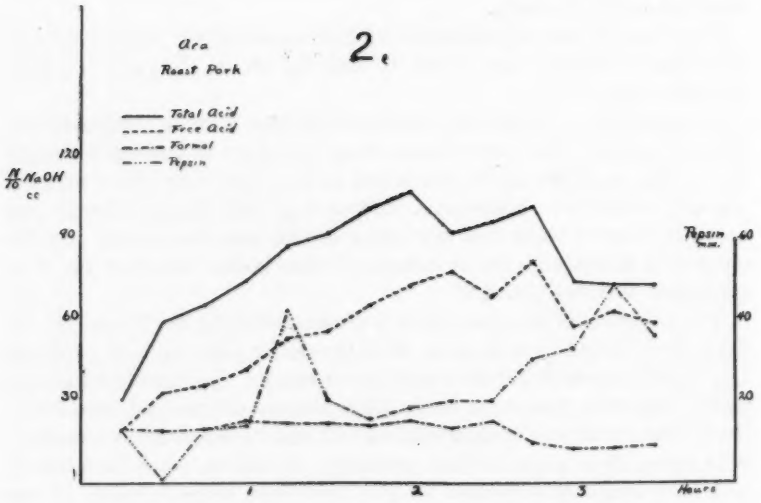
Figures 13 and 24 illustrate the digestion of pork chops. Compare figure 24 with figure 4 showing digestion of roast beef and note that practically the same time was required for each. In the same way compare figures 13 and 27 in the article on beef (1), noting the markedly longer time required for pork in the case of this individual. Pork chops and roast pork appear to require about the same periods for digestion in the stomach.

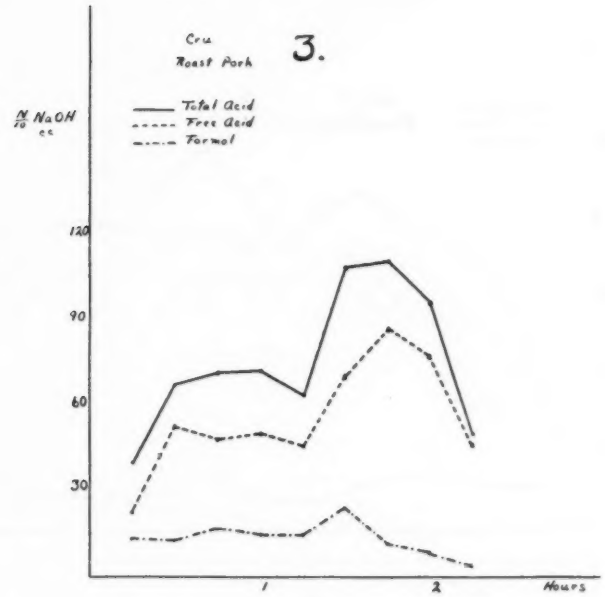
Fried ham in our experiments required considerably longer to digest than beef (compare figs. 5 and 6, also fig. 20 in the paper on beef products (1)).

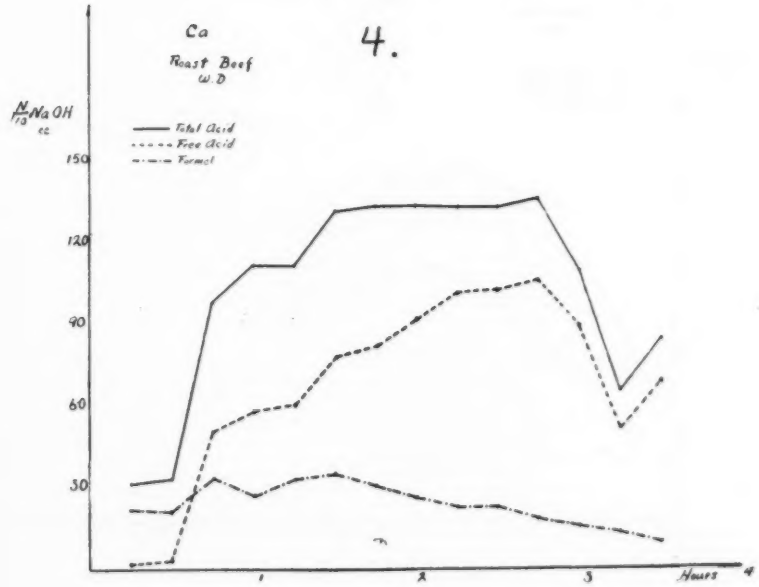
A comparison of boiled ham and minced ham may be obtained from figures 7 and 8. The minced ham shows but slight advantage although the acidity is more rapidly developed in this case than where mincing was not employed. Likewise a difference of only fifteen minutes was noted in favor of roast beef as compared with the minced ham or a difference of thirty minutes as compared with boiled ham (see fig. 9 as compared with figs. 7 and 8).

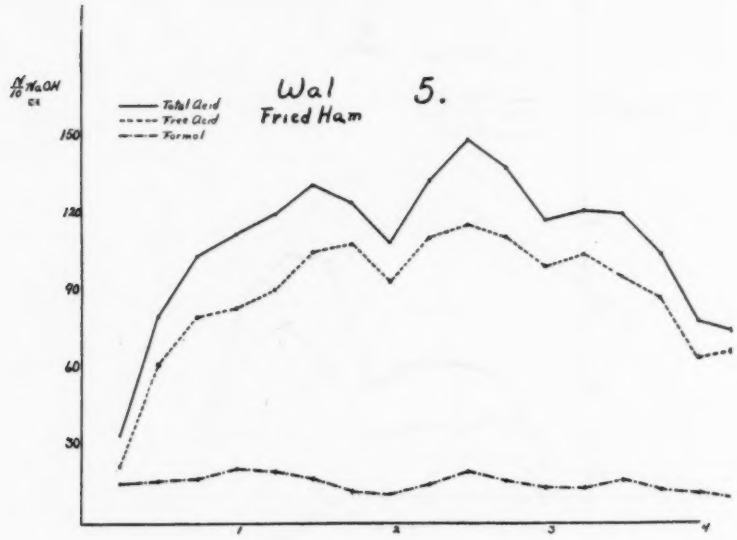
The digestion of liver and bacon is illustrated by figures 10 and 11. If figure 10 is compared with figure 22 of the earlier paper on beef products (1), it will be noted that liver and bacon require appreciably longer for gastric digestion than roast beef. Comparisons of liver and bacon with liver alone are shown by figures 11 and 12, and by figure 10 as compared with figure 25 in paper on beef products. It will be noted that from $\frac{1}{2}$ to 1 hour longer was required for the fried liver without bacon in one case while in the other case the bacon slightly prolonged the evacuation time.

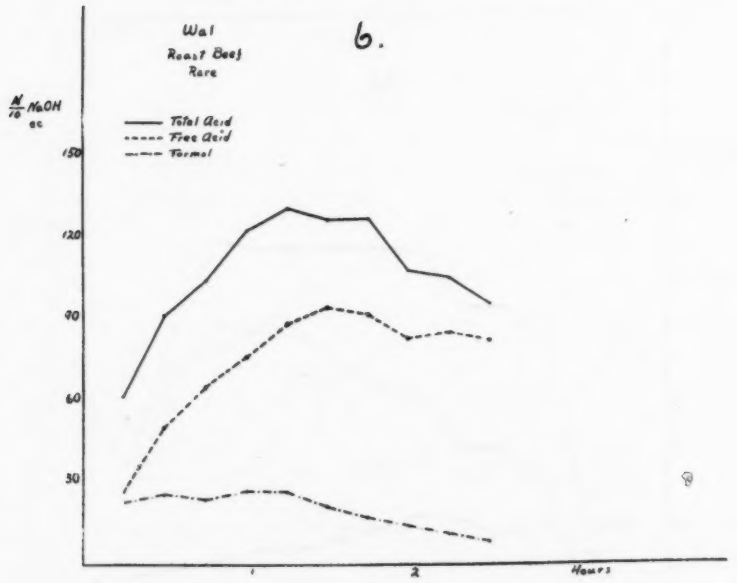

Spn
Roast Pork
1.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours
4

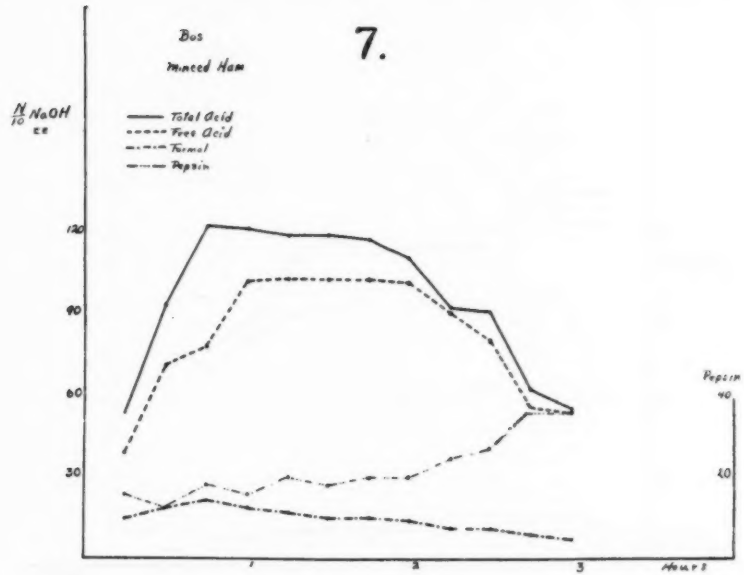

Ara
Roast Pork
2.
Total Acid
Free Acid
Formol
Pepsin
N/10 NaOH cc
120
90
60
30
1
Pepsin mm.
60
40
20
1
2
3
Hours

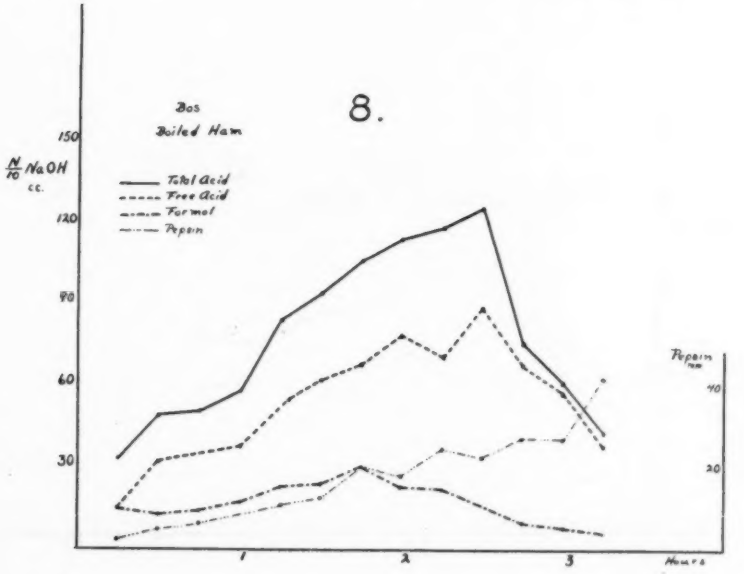

Cru
Roast Pork
3.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
Hours

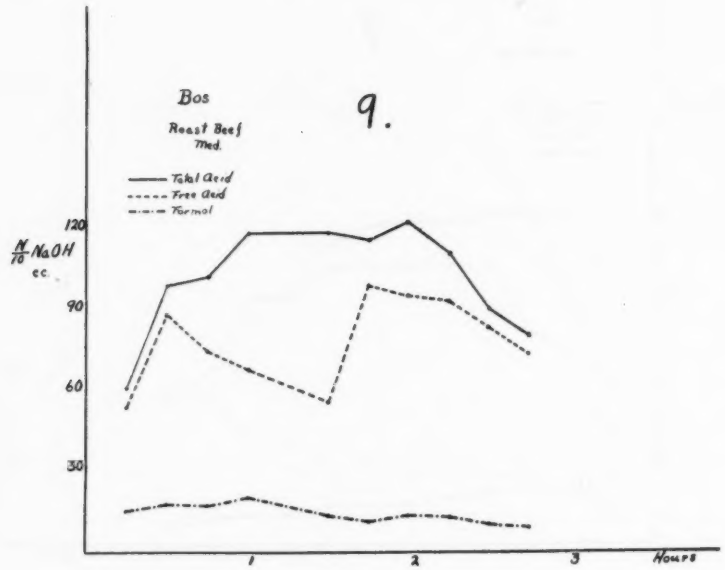

Ca
Roast Beef
W.D
4.
N/10 NaOH cc
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
Hours
4

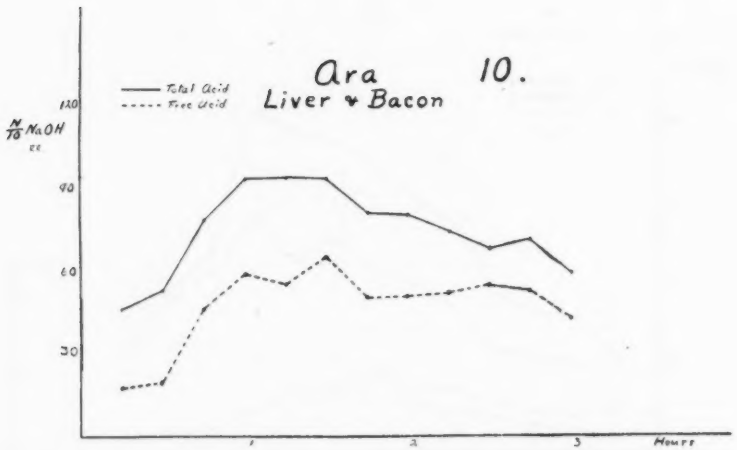

N/10 NaOH cc
Total Acid
Free Acid
Formol
Wal
Fried Ham
5.
150
120
90
60
30
1
2
3
4

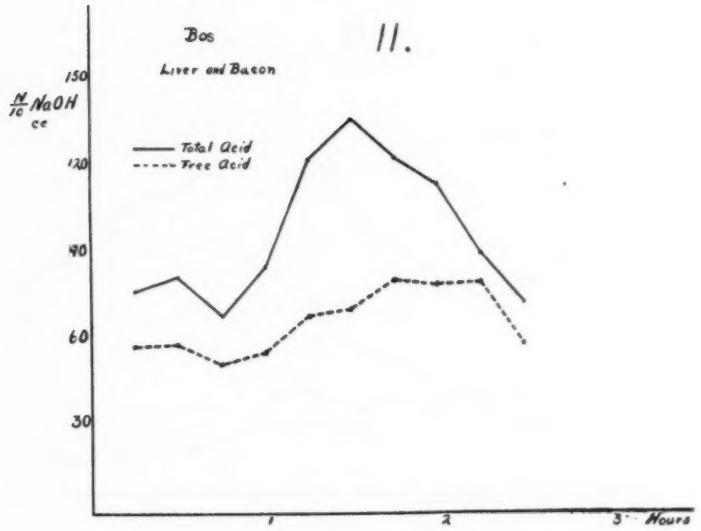

Wal
Roast Beef
Rare
6.
N/10 NaOH cc
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
Hours

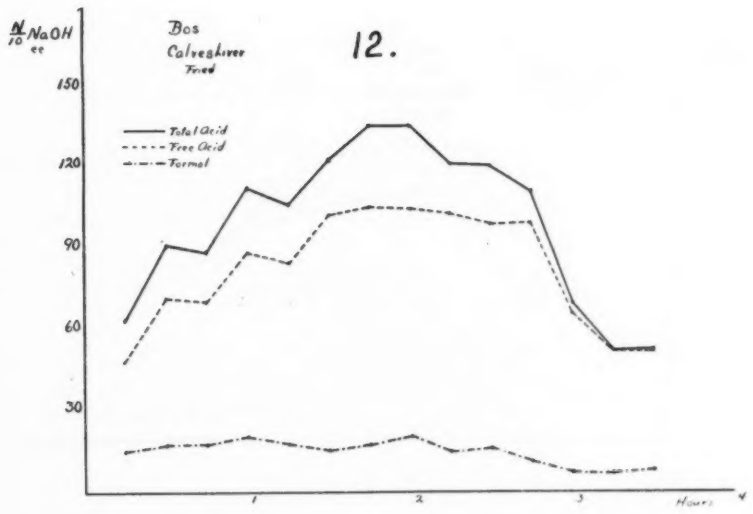

Bos
Minced Ham
7.
N/10 NaOH cc
Total Acid
Free Acid
Formol
Pepsin
120
90
60
30
Pepsin
40
20
1
2
3
Hours

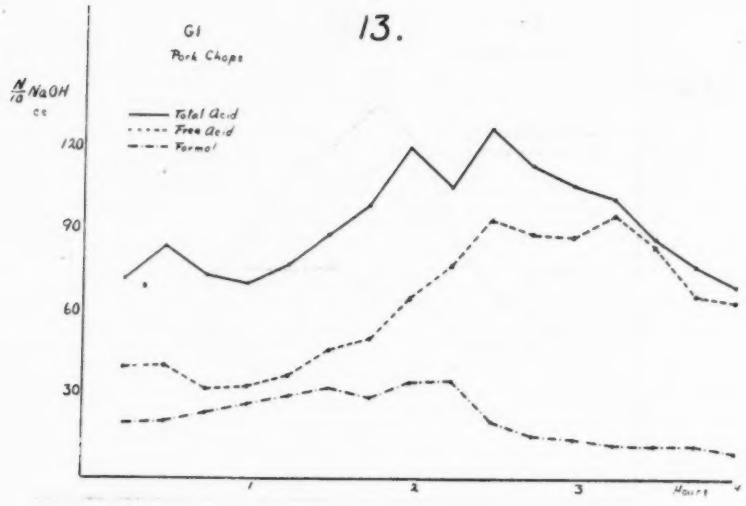

Bos
Boiled Ham
8.
N/10 NaOH cc.
Total Acid
Free Acid
Formol
Pepsin
150
120
90
60
30
Pepsin mm
40
20
1
2
3
Hours

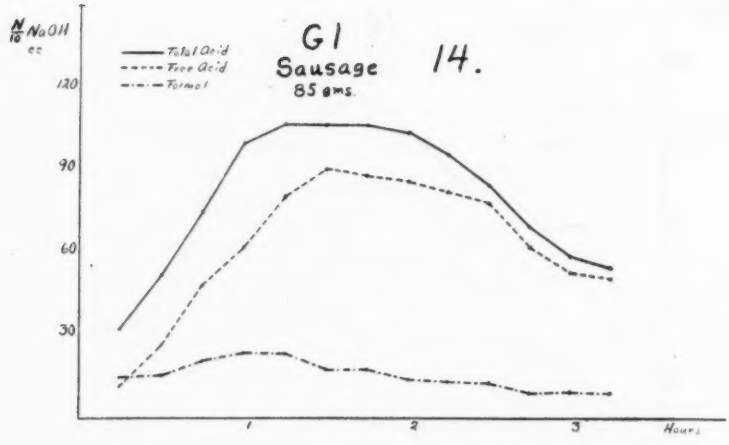

Bos
Roast Beef
Med.
9.
Total Acid
Free Acid
Formol
120
N/10 NaOH
cc.
90
60
30
1
2
3
Hours

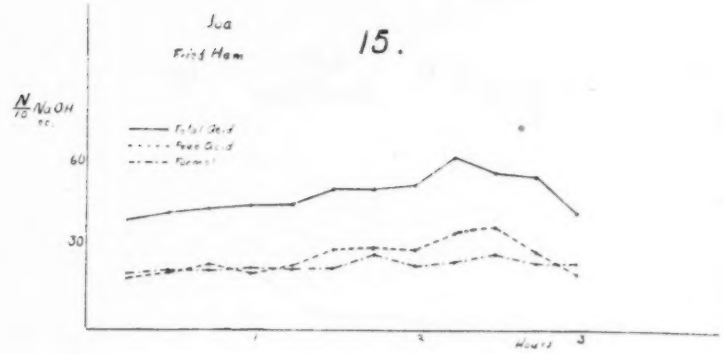

Ara
Liver & Bacon
10.
Total Acid
Free Acid
120
N/10 NaOH
cc
90
60
30
1
2
3
Hours

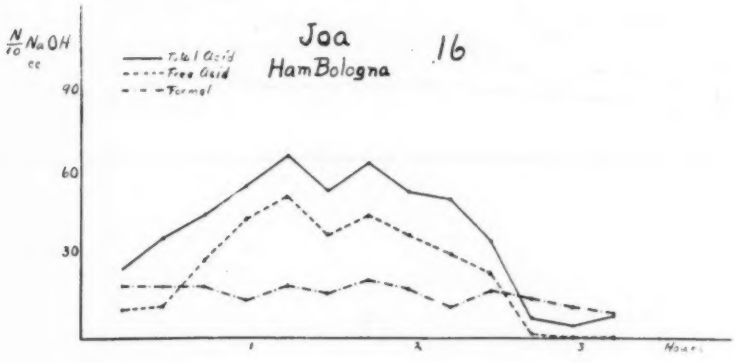

Bos
11.
Liver and Bacon
N/10 NaOH cc
150
120
90
60
30
Total Acid
Free Acid
1
2
3
Hours

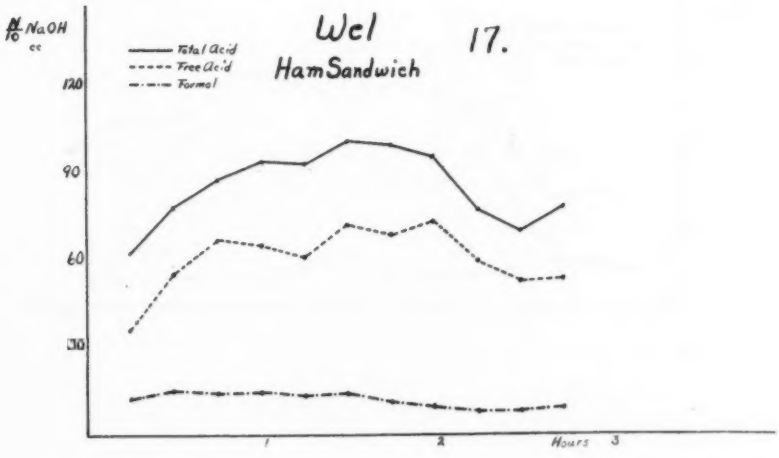

N/10 NaOH cc
Bos
Calves liver
Fried
12.
150
120
90
60
30
Total Acid
Free Acid
Formol
1
2
3
4
Hours

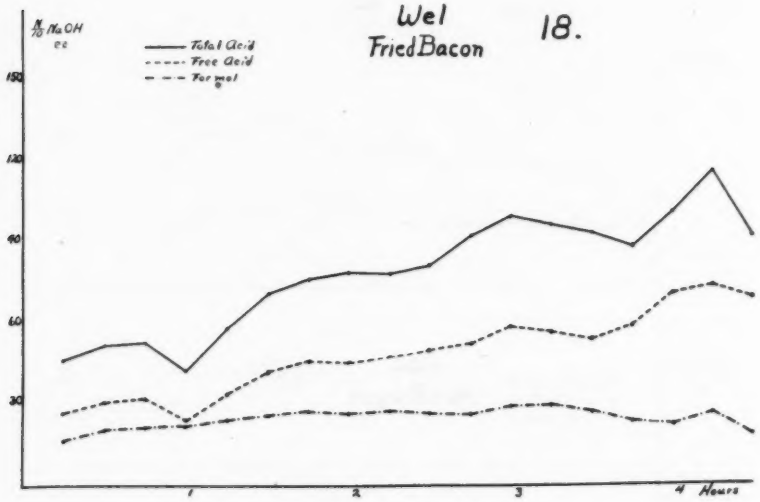

G1
Pork Chops
13.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
3
Hours

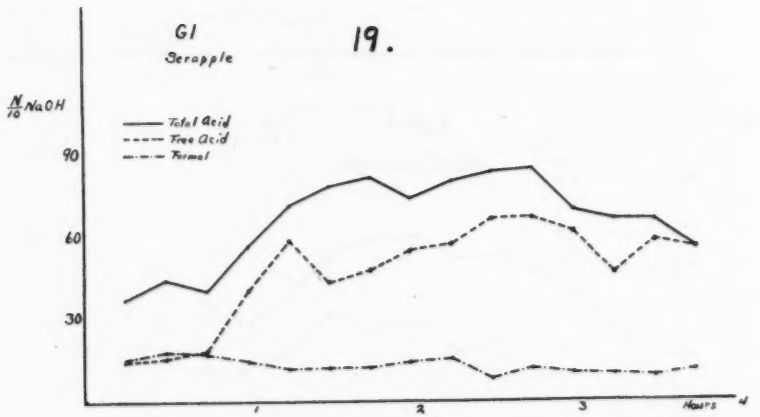

N/10 NaOH cc
Total Acid
Free Acid
Formol
G1
Sausage
85 gms.
14.
120
90
60
30
1
2
3
Hours

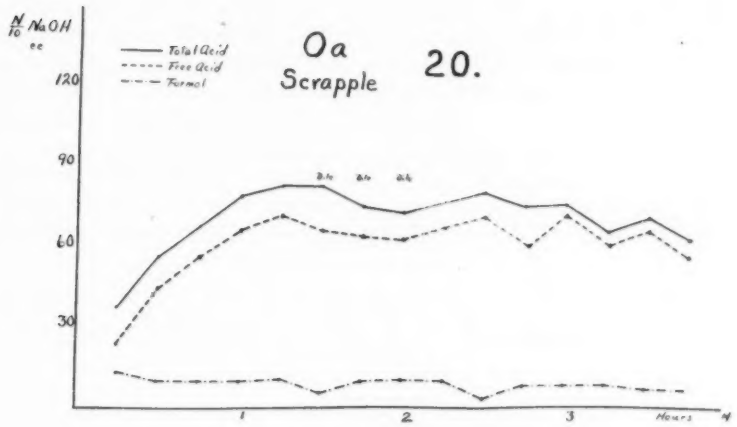

Jua
Fried Ham
15.
N/10 NaOH cc.
Total Acid
Free Acid
Formol
60
30
1
2
Hours
3

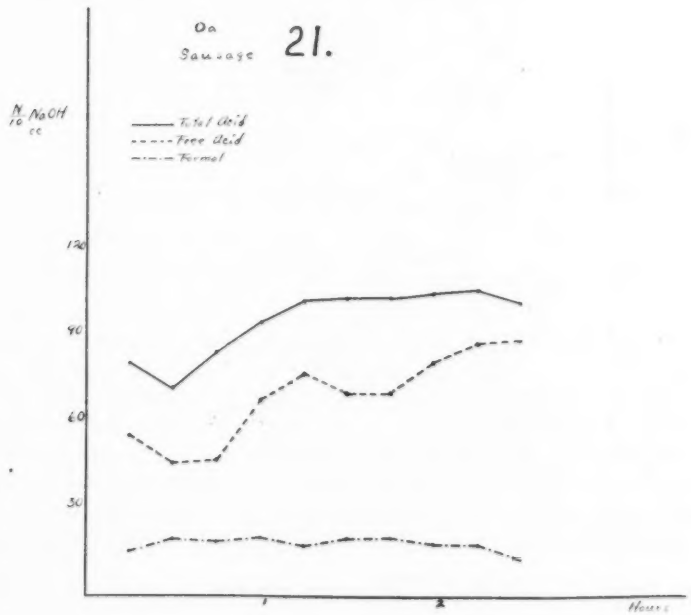

N/10 NaOH cc
Total Acid
Free Acid
Formol
Joa
Ham Bologna
16
90
60
30
1
2
3
Hours

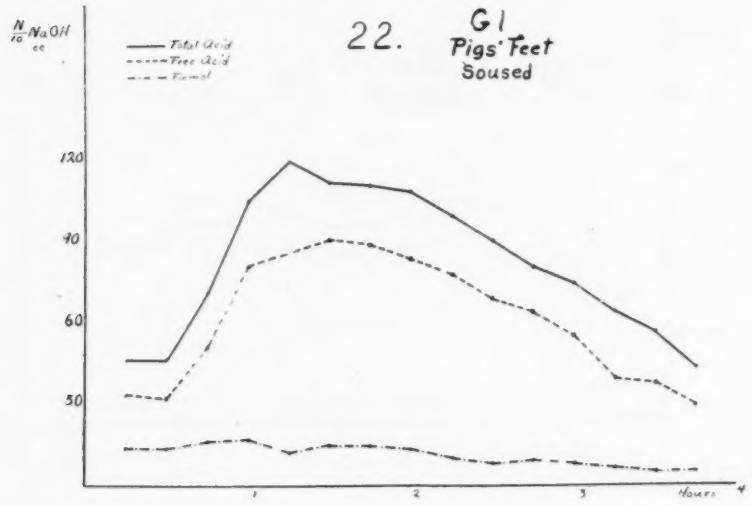

N/10 NaOH cc
Total Acid
Free Acid
Formol
Wel
Ham Sandwich
17.
120
90
60
30
1
2
Hours
3

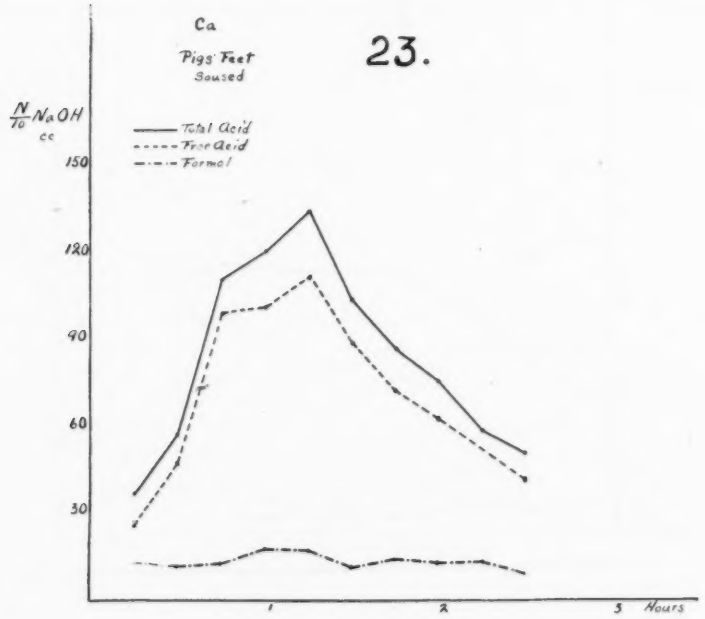

N/10 NaOH cc
Total Acid
Free Acid
Formol
Wel
Fried Bacon
18.
150
120
90
60
30
1
2
3
4 Hours


GI
Scrapple
19.
N/10 NaOH
Total Acid
Free Acid
Formol
90
60
30
1
2
3
Hours
4


N/10 NaOH cc
Total Acid
Free Acid
Formol
Oa
Scrapple
20.
120
90
60
30
1
2
3
Hours
4

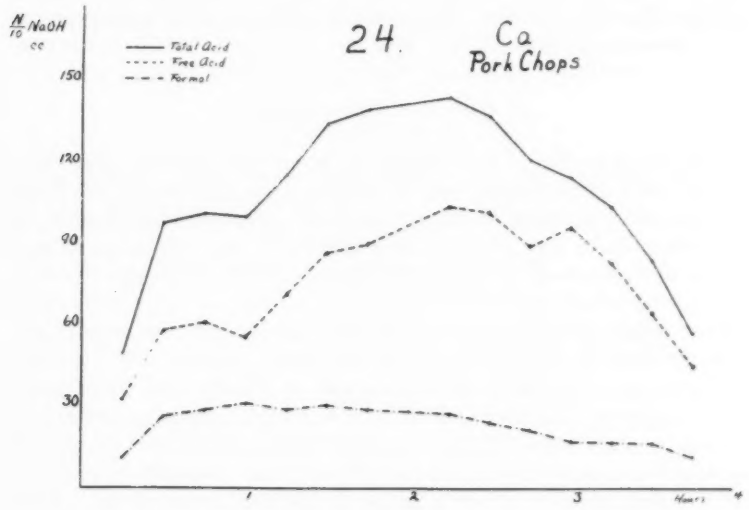

Oa
Sausage
21.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
2
Hours


N/10 NaOH cc
Total Acid
Free Acid
Formol
22. G1
Pigs' Feet
Soused
120
90
60
30
1
2
3
Hours
4


Ca
Pigs' Feet
Soused
23.
N/10 NaOH cc
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3 Hours

The comparative responses of pork sausage and pork chops are illustrated by figures 13 and 14. The averages given in table 3 also indicate that the sausage is somewhat more easily handled. Compare also with roast beef (fig. 27) and beef frankfurters (fig. 26, beef article) and note that the beef has an advantage over sausage.

A comparison of fried ham with ham bologna is given by figures 15 and 16, little difference being shown as to response. These charts should be compared with figures 5, 6, 7 and 8 in preceding paper on beef products. The results obtained indicate that ham remains in the



stomach somewhat longer than steaks of the tougher cuts and about the same time as a sirloin or tenderloin steak.

The slow evacuation of bacon when ingested in as large amounts as 100 grams is illustrated by figure 18. The comparatively slow development of acidity may also be noted. The high fat content is presumably responsible for these effects. In contrast with this note the fairly rapid evacuation where two ham sandwiches were given (100 grams bread and 23 grams boiled ham) (fig. 17), also the more rapid development of free acidity.

The so-called "scrapple" is a preparation made from pork and cereal, whose "habitat" is more or less restricted to certain localities. The

head and jowls of the pig are sometimes used as well as heart, liver and meat trimming. The cereals used most commonly are wheat flour, buckwheat flour and cornmeal mixtures. That scrapple leaves the stomach rather slowly is indicated by the charts (figs. 19 and 20) as compared with roast beef (fig. 27, beef article), pork chops (fig. 13) and sausage (fig. 14). The greater delay of digestion of "scrapple" as compared with pork sausage is shown also by figures 20 and 21.

Soused pigs' feet were also tested. In one case the pigs' feet required practically as long to leave the stomach as pork chops (compare figs. 22 and 13). In the other case the pigs' feet were digested much more rapidly (figs. 23 and 24). The latter type of response is perhaps the more characteristic.

SUMMARY AND CONCLUSIONS

A series of studies of the response of the normal human stomach to various pork products prepared in various ways was carried out using the fractional method of gastric analysis. The average results for evacuation times and acidities developed have been tabulated. Comparative responses of the same individuals to these various products as well as to certain beef products have been charted. For individuals with stomachs of the rapid-emptying type a general average evacuation time for pork products of $2\frac{3}{4}$ hours was found. Subjects of the slow-emptying type showed a general average of 3 hours and 40 minutes. The average total acidity observed at the height of digestion was 117.

Pork products in general were comparatively slow to leave the stomach as would be expected from their high fat content. The differences were not as great, however, as some figures in the literature indicate. Thus roast pork was retained appreciably longer than roast beef in most instances. Pork chops required about the same period of gastric digestion as roast pork. Fried ham also required considerably longer to digest than roast beef.

Minced ham showed a slight advantage over boiled ham as to evacuation time. Acidities were also more rapidly developed in the former instance. Roast beef was handled somewhat more quickly than either.

Liver and bacon required about the same period of digestion as roast beef. In certain instances liver and bacon were handled more readily than liver alone. In one case the contrary was found.

Pork sausage was somewhat more easily handled than were pork chops but less readily than roast beef.

Ham bologna required about the same time to digest as fried ham or as the less readily digestible beef steaks.

The evacuation of bacon was found to be slow and low gastric acidities were developed. Ham sandwiches were more readily handled than most other pork products tested.

"Scrapple" left the stomach more slowly than pork sausage and belongs to the less readily evacuated pork products.

Pigs' feet gave variable results but appear ordinarily to be handled more easily than pork chops.

The authors wish to extend their thanks to Dr. R. A. Lichtenthaeler who assisted them in carrying out these tests and also to those Jefferson men who sacrificed time and convenience in acting as subjects.

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(2) Beaumont: Physiology of digestion, 2nd Ed., 1847, Burlington, Vt.
(3) Jessen: Zeitschr. f. Biol., 1883, xix, 129.
(4) Penzoldt: Deutsch. Arch. klin. Med., 1893, li, 535.

GASTRIC RESPONSE TO FOODS[1]

V. The Response of the Stomach to Lamb and Lamb Products

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The present series of tests on the response of the normal human stomach to lamb and lamb products was carried out in the same manner as our experiments on beef (1) and pork (2) products and with a similar object. Beaumont (3) reported on his subject an evacuation time of $3\frac{1}{4}$ hours for roast lamb as compared with 3 to $3\frac{1}{2}$ hours for roast beef. Jessen (4) reports two experiments with raw lamb as compared with raw beef and found both to leave the stomach in two hours.

In the present paper are reported the results of fourteen tests with roast lamb and other lamb products on several different normal subjects as well as certain tests on beef and pork products for direct comparison. One hundred grams of meat were ingested in each case.

The results of these experiments are averaged in table 1. Values obtained for men whose stomachs in general empty rapidly are given separately from the values obtained with men whose stomachs were of the slow-emptying type. The distinction between the two types of stomach is not so marked in the case of lamb products as it is with certain other foods. As the table indicates from $2\frac{1}{2}$ to $2\frac{3}{4}$ hours were required for lamb to leave the stomach in the one case and 3 to 4 hours in the other.

The charts showing the results of fractional gastric analyses in a number of cases where these meats were fed enable us to compare the responses of the same individual to various meats. In certain cases reference is made to preceding papers upon beef and pork products for such comparisons. The acid curves enable us to distinguish between

[1] The expenses of this investigation were defrayed by funds furnished by Mrs. M. H. Henderson, the Curtis Publishing Company and Dr. L. M. Halsey.

certain pathological responses where evacuation is rapid although little acid is secreted and hence little gastric digestion occurs, and a normal type of rapid-emptying stomach where acid secretion is normal and considerable protein digestion takes place in this organ.

The digestion of roast lamb is illustrated by figures 1 to 5. The extreme variation in evacuation times of these normal subjects (compare, for example, figure 1 showing an evacuation time of $3\frac{3}{4}$ hours with

TABLE 1

Gastric digestion of lamb and lamb products

NUMBER	SUBJECT	MEAT	TYPE OF STOMACH							
			Rapid-emptying				Slow-emptying			
			Evacuation time, hours and minutes		Highest total acidity*		Evacuation time, hours and minutes		Highest total acidity	
				average		*average*		*average*		*average*
1	Riv	Roast lamb	1:45		114					
2	Ara	Roast lamb	3:15		138					
3	Bos	Roast lamb	3:45		147					
4	Don	Roast lamb	2:30		147					
5	Han	Roast lamb	2:45	2:45	142	137				
6	Wel	Roast lamb					3:45		156	
7	Cop	Roast lamb					3:00	3:30	123	140
8	Cop	Stewed lamb					4:00		122	
9	Ber	Stewed lamb					4:00	4:00	150	136
10	Tri	Lamb chops	2:30	2:30	140	140				
11	Cop	Lamp chops					3:30		115	
12	Oa	Lamb chops					2:30	3:00	115	115
13	Hou	Sheep brains					2:15		97	
14	Sim	Sheep brains					2:45	2:30	73	85

*Acidities are expressed in terms (cc.) of N/10 alkali required to neutralize 100 cc. of sample.

figure 3 where the stomach is emptied in $1\frac{1}{2}$ hours) is of interest. The subject of experiment 3 is however unusual in this respect.

For comparisons of roast lamb with roast beef, observe figure 1 showing an evacuation time of $3\frac{3}{4}$ hours and figure 9 in preceding paper on pork products which shows an evacuation time for roast beef of $2\frac{3}{4}$ hours. Also compare figure 3 showing an emptying time of $1\frac{1}{2}$ hours with experiments on roast beef with the same individual recorded in preceding paper on beef products (figs. 1 and 2) which show the beef leaving in 2 hours. A more marked difference in favor of roast beef is

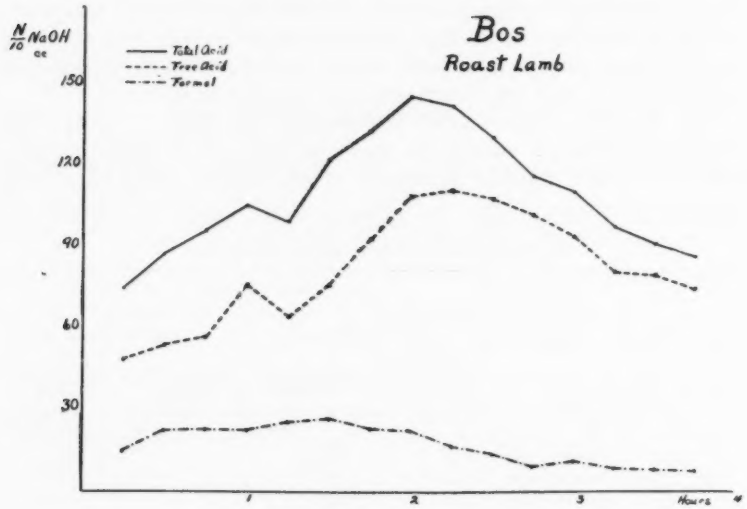
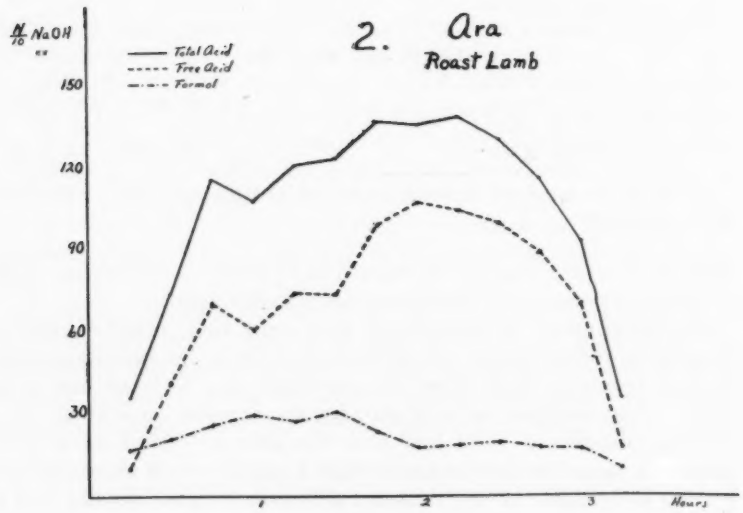
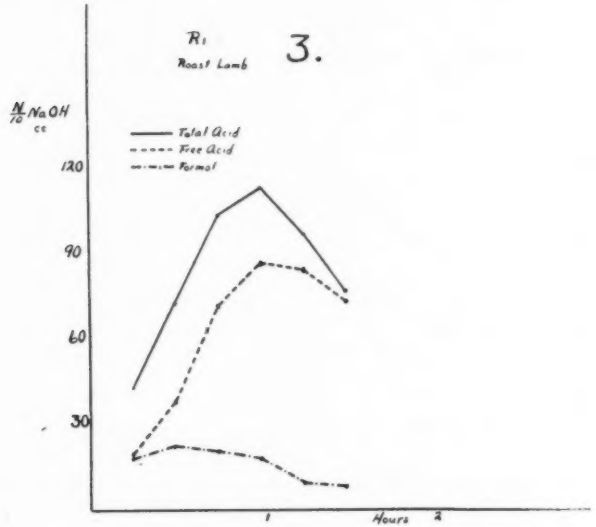
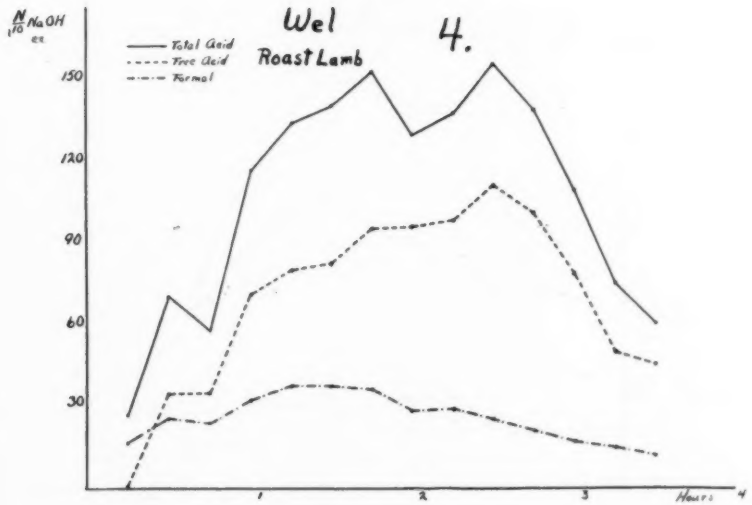
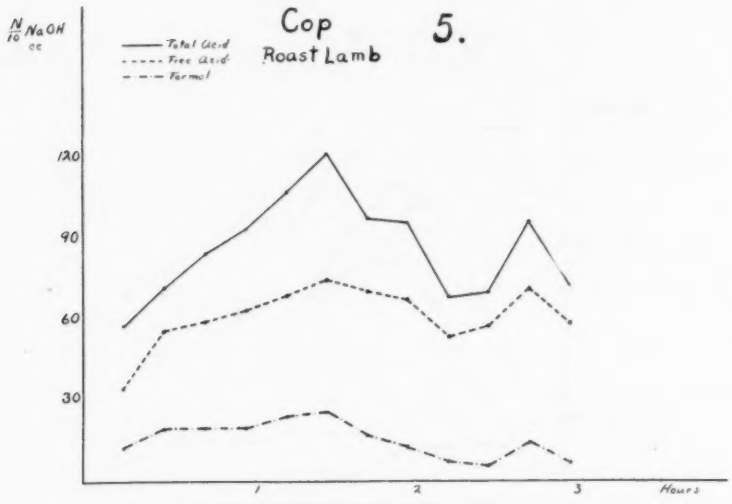

N/10 NaOH cc
Total Acid
Free Acid
Formol
Bos
Roast Lamb
150
120
90
60
30
1
2
3
Hours

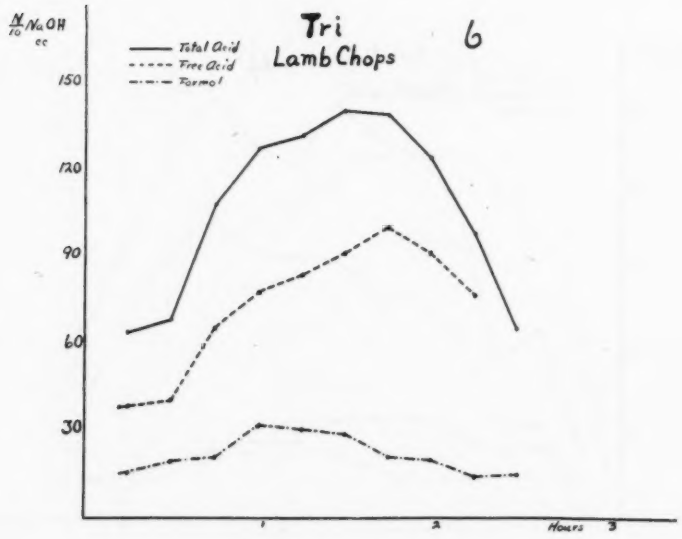
Fig. 1


N/10 NaOH cc
Total Acid
Free Acid
Formol
2. Ara
Roast Lamb
150
120
90
60
30
1
2
3
Hours


R1
Roast Lamb
3.
N/10 NaOH cc
Total Acid
Free Acid
Formol
120
90
60
30
1
Hours
2


N/10 NaOH cc
Wel
Roast Lamb
4.
Total Acid
Free Acid
Formol
150
120
90
60
30
1
2
3
Hours
4


N/10 NaOH cc
Total Acid
Free Acid
Formol
Cop
Roast Lamb
5.
120
90
60
30
1
2
3
Hours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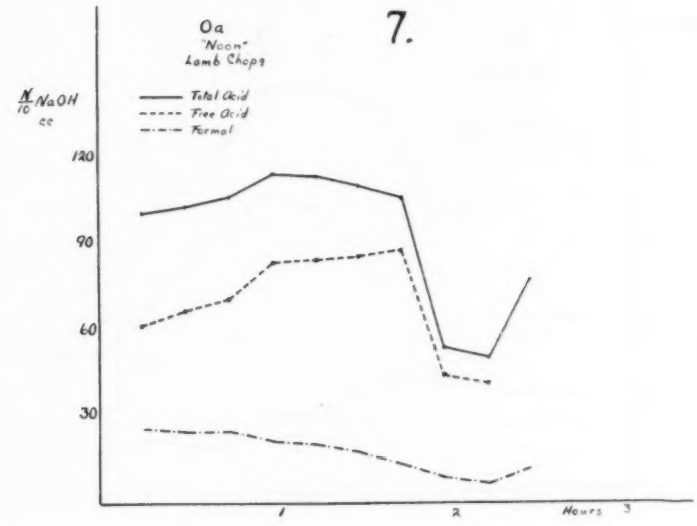
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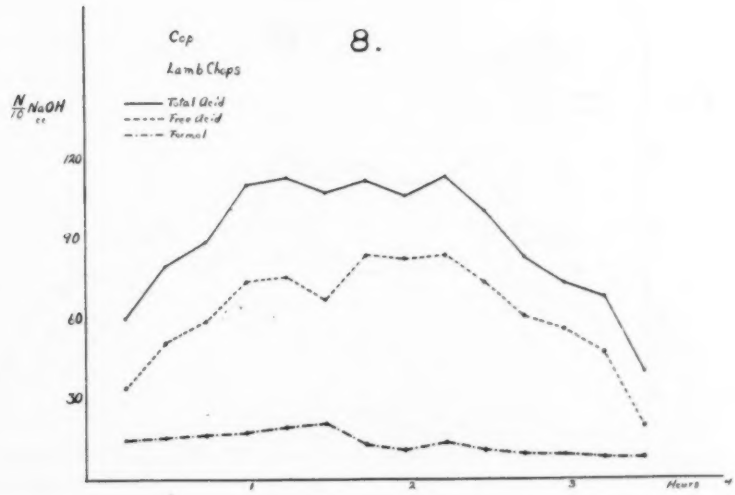
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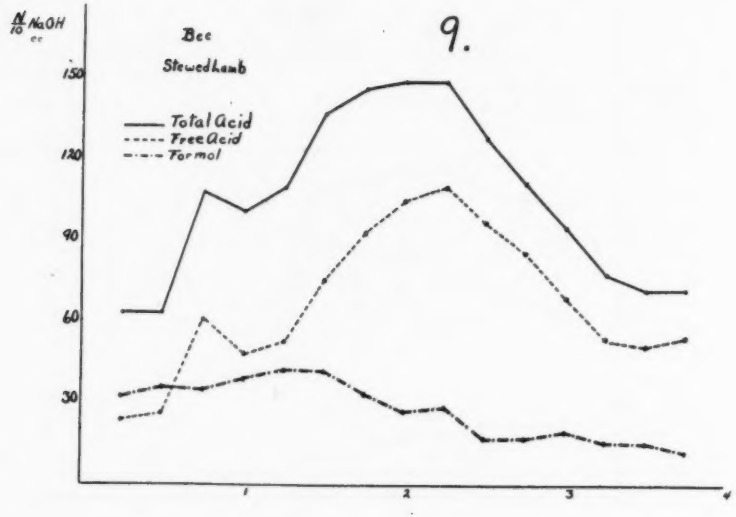
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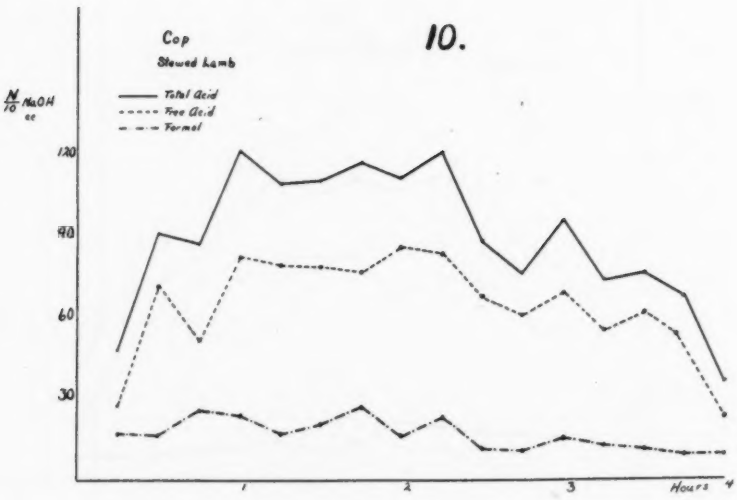
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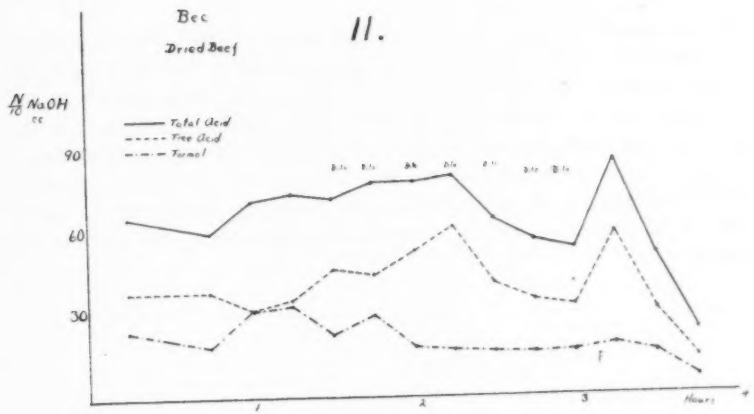
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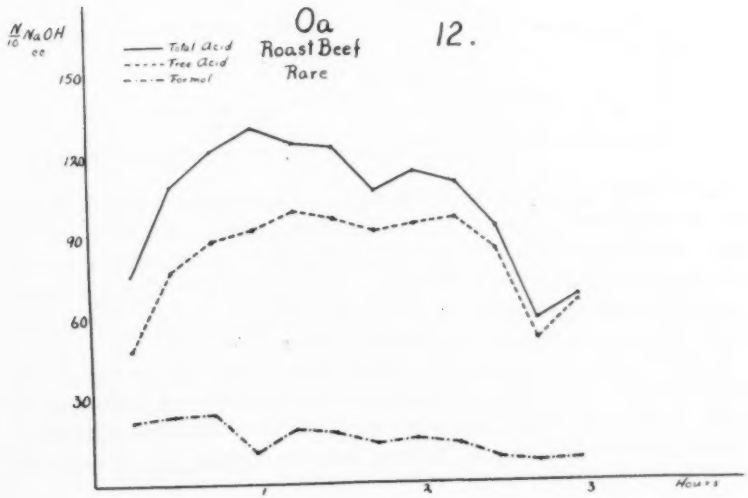
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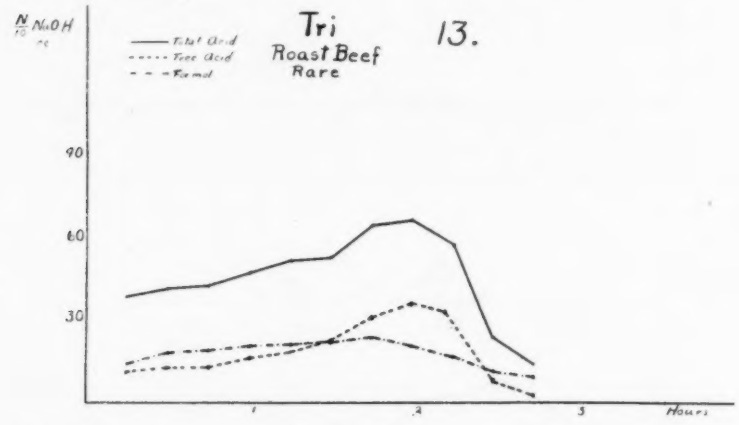
shown by figure 2 as compared with figure 22 in article on beef, the latter leaving in $1\frac{3}{4}$ hours.

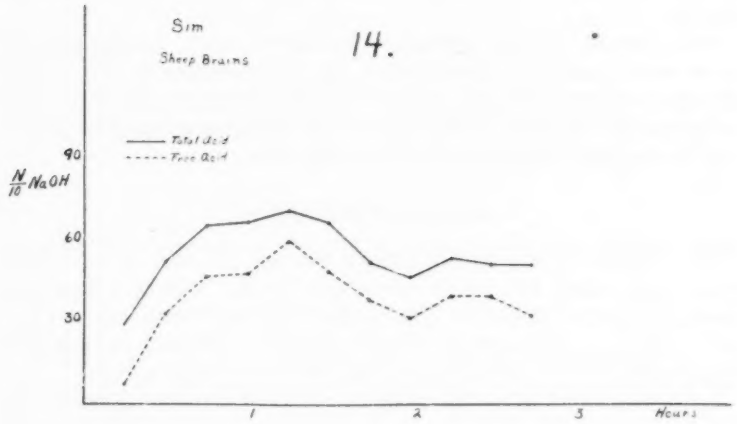
A comparison of roast lamb and roast pork is obtained from figure 2 and figure 12 in preceding paper on pork products, the roast pork requiring $3\frac{1}{2}$ hours digestion as compared with $3\frac{1}{4}$ for lamb chops. Roast lamb may also be compared with boiled ham, figure 1 showing evacuation time for roast lamb of $3\frac{3}{4}$ hours while figure 8 in preceding article on pork shows $3\frac{1}{4}$ hours for the boiled ham. As would be expected, roast lamb (fig. 4, $3\frac{1}{2}$ hours) leaves the stomach sooner than bacon (fig. 18 in preceding article on pork showing evacuation time of $4\frac{1}{2}$ hours).



Lamb chops appear to require about the same period of gastric digestion as roast lamb (see table 1). A direct comparison of the two on the same individual may be obtained from figure 5 (roast lamb, 3 hours) and figure 8 (lamb chops, $3\frac{1}{2}$ hours). Compare also figure 6, lamb chops, $2\frac{1}{2}$ hours, with figure 13, roast beef, $2\frac{3}{4}$ hours and also with rump steak, $2\frac{3}{4}$ hours (figure 10 in preceding paper on beef).

Further comparisons of lamb chops with roast beef are given by figure 7, lamb chops, $2\frac{1}{2}$ hours and figure 12, roast beef, 3 hours and by figure 31 in beef article, sirloin steak, 3 hours. The same individual shows an evacuation time of $2\frac{1}{2}$ hours for pork sausage (fig. 21 in article on pork).

Stewed lamb (fig. 10) left the stomach in 4 hours as compared with $3\frac{1}{2}$ hours for lamb chops (fig. 8) and 3 hours for roast lamb (fig. 5). In


Sim
Sheep Brains
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Total Acid
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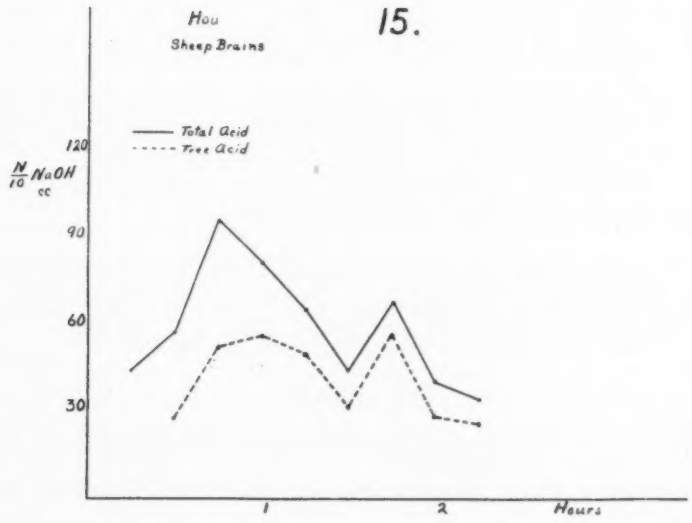
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Sheep Brains
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Hours

another case (fig. 9) stewed lamb required the same time as dried beef (fig. 11).

Sheep brains left the stomach in moderate time. In one case $2\frac{3}{4}$ hours were required for sheep brains (fig. 14) as compared with $3\frac{1}{4}$ and $3\frac{1}{2}$ hours for roast beef (figs. 3 and 4 in preceding paper on beef). An individual who showed an evacuation time of $2\frac{1}{2}$ hours for sheep brains (fig. 15) required 3 hours for raw hamburg steak.

SUMMARY AND CONCLUSIONS

The response of the normal human stomach to roast lamb, stewed lamb, lamb chops and sheep brains was studied using the fractional method of gastric analysis. The results have been tabulated as averages (table 1). Comparisons of lamb cooked in different ways as well as comparisons of lamb with pork and beef have been charted.

Lamb was found to require from 2 to 3 hours (average $2\frac{1}{2}$ hours) for individuals possessing the rapid-emptying type of stomach to digest and from 3 to 4 hours (average 3 hours 20 minutes) for individuals with the slow-emptying type of stomach. Roast lamb and lamb chops required practically the same period of gastric digestion and stewed lamb a little longer than the other two. Sheep brains left the stomach rather rapidly ($2\frac{1}{2}$ hours) and developed a lower acidity than the other meats.

Lamb stimulated acid production fully as much as any other class of meats and apparently to a slightly greater extent than beef or pork. The average total acidity at the height of digestion was 134.

On the average, roast lamb remained in the stomach a few minutes longer than roast beef but not as long as roast pork.

The authors wish to extend their thanks to Dr. R. A. Lichtenthaeler who assisted them in carrying out these tests, and also to those Jefferson men who sacrificed time and convenience in acting as subjects.

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THE ELECTRICAL CONDUCTIVITY METHOD OF DETERMINING THE RELATIVE VOLUME OF CORPUSCLES AND PLASMA (OR SERUM) IN BLOOD

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Many years ago (1) I worked out a method of determining the percentage volume of plasma (or serum) in blood, based upon the fact that the corpuscles may be assumed to be non-conducting particles suspended in the plasma.

Although the method unquestionably gives more accurate results than the haematocrite and requires little time, it has not been greatly employed even in the laboratory, still less in the hospital. P. Fraenkel (2) recommended it for scientific purposes, after comparing it with Bleibtren's chemical method, and Wilson (3) compared it with the haematocrite method. However, he did not keep up the rotation of the haematocrite until the column of corpuscles had assumed a constant volume, but rotated for a fixed time (five minutes). This was done purposely because frequently the haematocrite is employed in this way. But although useful comparative results may be obtained on the same blood with a fixed time of rotation, this is not the case where bloods differing greatly in the percentage of corpuscles are to be compared.

As the measurement of the conductivity of physiological liquids is a much more familiar operation among biologists and clinicians than it was twenty years ago, and as we have been recently making use of the method again in calculating epinephrin concentrations in serum from the concentrations in the corresponding blood, and have had the opportunity to compare it rather extensively with the haematocrite method, I am confirmed in the belief that its advantages have not been sufficiently appreciated. To be sure, a larger quantity of blood is needed than that required to fill a haematocrite tube. I use a conductivity tube holding about 3 cc. and, therefore, must have 3 cc. of serum. But it is not difficult, as Wilson did, to employ a tube which requires only 4 to 5

drops of blood. The principle of the method permits the assumption that the formulae worked out on dog's blood will also be applicable to other bloods, since the corpuscles can always be taken as practically non-conducting.

In the previous paper the percentage volumes of serum as determined by the electrical method in thirty dogs taken at random are given. The range is from 39.6 to 74.0, and the average 55 per cent. The average for blood specimens taken from six of the dogs used in the recent work is 52 per cent of serum, the range 36.5 to 66.5. The results are compared with haematocrite readings in table 1.

TABLE 1

NUMBER OF ANIMAL	PERCENTAGE OF SERUM BY	
	Electrical method	Haematocrite
1	52.3	48 (5 minutes), 49 (10 minutes), 50 (15 minutes)
2	36.5	29.5 (40 minutes), 33 (60 minutes), 35 (70 minutes)
263	66.5	62 (15 minutes), 65 (23 minutes)
297	51.0	25.5 (10 minutes), 36 (20 minutes), 41 (30 minutes), 45 (40 minutes) 48 (50 minutes)
306	46.6	10 (10 minutes), 25 (20 minutes), 33 (32½ minutes)
307	62.0	

It will be observed that while great differences exist between the haematocrite readings with a given blood, according to the time of rotation, in no case does the percentage of serum with the longest time of rotation quite equal the percentage determined by the electrical method. The latter obviously constitutes a limit toward which the haematocrite readings approach more nearly the longer the rotation is continued. With some bloods the approach is extraordinarily slow (dogs 2 and 306, e.g.). Where the serum is scanty this is usually the case. In dog 306 after 32½ minutes rotation the end point had not been nearly reached. The percentage of serum as determined by the haematocrite at this time would have been less than two-thirds of the real percentage. The speed of the haematocrite was about 4000 turns a minute. A very high speed was purposely avoided so as to permit such differences to be readily detected. But with higher speeds they would still exist, although the end point would be reached sooner.

In table 2 is given a similar comparison on bloods from eighteen cats.

TABLE 2

NUMBER OF ANIMAL	PERCENTAGE OF SERUM BY	
	Electrical method	Haematocrite
1		73 (5 minutes), 73.5 (10 minutes), 74 (15 minutes), 74.5* (20 minutes)
2	62.5	57 (8 minutes), 60.5 (16 minutes), 62 (21 minutes)
8		71 (5 minutes), 71.5 (10 minutes), 72* (15 minutes)
9		51.5 (5 minutes), 53.5 (10 minutes), 54.5 (15 minutes), 55* (20 minutes)
91		87*†
214	71.0	
239	70.0	
259	76.5	65 (8 minutes), 70 (15 minutes), 73.5 (20 minutes)
284		50.5 (15 minutes), 51 (22 minutes), 52 (34 minutes)
285	56.5	46 (15 minutes), 49.5 (22 minutes,) 52 (34 minutes)
286	67.3	32 (5 minutes), 57 (15 minutes), 60 (22 minutes), 62 (30 minutes)
287	44.7	30 (5 minutes), 40 (15 minutes), 41 (22 minutes), 42 (30 minutes)
288	67.1	
289	72.3	69 (15 minutes), 70 (25 minutes)
290	57.0	53 (10 minutes), 56 (20 minutes)
298	82.0	
305	53.8	43 (10 minutes), 48.5 (20 minutes), 51.5 (32½ minutes)
308		65 (10 minutes), 68 (15 minutes), 69.5 (20 minutes) 70* (25 minutes)

* With these bloods rotation was continued until the increment of the column of serum in successive rotation periods became negligibly small. It was seldom that an absolutely constant length was reached.

† This blood as it sedimented in the test tubes obviously consisted mainly of serum.

The average for the eighteen cats, including those where only haematocrite determinations were made, is 66.2 per cent of serum, the range 44.7 to 87 per cent. The average for all the cats used by us would certainly be distinctly higher, for a number of old animals with an abnormally low serum content for a cat are included in the table.

In accordance with the fact that the cat's corpuscles in general sediment much more rapidly on standing than the dog's, and also because of the lower proportion of corpuscles, the haematocrite readings approximate sooner to the percentage determined electrically. The latter still, however, constitutes a limit which is not exceeded by the haematocrite determinations. I have found the same to be true for human blood, for example, in a case of diabetes insipidus with anaemia, studied along with Christie (4). Wilson (3) invariably obtained lower serum

percentages by the haematocrite than by the electrical method in pathological cases. Probably the difference in these cases would have been reduced by longer centrifugalization. That the electrical method gives results which are approximately correct was established by comparison with two other methods in the former paper (1). It is strong corroborative evidence that the haematocrite readings come nearer and nearer to the values determined by the electrical method, the longer the rotation, without quite attaining them, since some serum is inevitably left in the sediment. In the electrical method no alteration whatever can be produced in the corpuscles, the measurement being made while they are normally suspended in the serum.

Since the viscosity of blood is influenced greatly by its content of corpuscles, it is easy to see that very considerable differences may exist in the coefficient of viscosity of the blood of healthy individuals of the same species and in the average viscosity of the blood of different kinds of animals. Burton-Opitz has shown this in his elaborate investigations on the viscosity of blood. He found that the viscosity of dog's blood was on the average five times greater than that of distilled water (at 37°C.), that of rabbit's blood only 3.4 times greater, while cat's blood possessed an intermediate value.

Lewy (6) also observed a great difference in the viscosity of blood from different animals of the same species as well as in animals of different species. Welsh (7) found a considerable range in healthy human beings and of course very great variations in disease.

The fact that great variations in the viscosity are compatible with a perfectly efficient circulation, suggests that the emphasis which has been placed on the superiority of such substances as gum or gelatine as constituents of transfusion liquids is scarcely warranted. In so far as they may attract water from the tissues and retain it in the circulation, they may have some advantage. But when it is argued that since gum or gelatine solutions by increasing or maintaining the viscosity of the blood enable a greater arterial pressure to be attained, they must be superior to simple salt solutions, the physiological basis for the conclusion seems open to question.

Why should it be necessarily advantageous to artificially increase the resistance which the heart must overcome in order to drive blood through the tissues? When the viscosity is increased the resultant rise of blood pressure, provided the response of the heart is adequate, does not mean as a matter of course that the tissues are getting more blood than before but may merely mean that the heart by working harder

is able to deliver to them as much blood as before. It is true that the physiologist or the physician who is estimating the blood pressure may have the satisfaction of seeing it mount toward what is considered a normal level, but beyond this it is not clear that there would be any necessary advantage. If the response of the heart is inadequate and the rise of blood pressure is insufficient to overcome the extra resistance, the blood flow in the tissues may be diminished although the pressure has been increased. Of course, it may be argued that a certain minimum blood pressure is essential and that if this were not reached the organs, including the heart, would not function properly, even if a sufficient blood flow through them could be maintained. This, however, is one of the moot points of physiology, while there is general agreement that the important thing is a sufficient flow of blood. There are certainly conditions in which this essential blood flow might be promoted by diminishing the viscosity of the blood. If no other change occurred the blood pressure might be expected to fall. But where the blood viscosity is diminished by the injection of salt solutions the volume of the circulating liquid is at the same time increased, and if the pressure does not fall (it is not necessary that it should rise) the blood flow will be increased. When blood corpuscles are injected the viscosity is necessarily increased and the flow to that extent rendered more difficult. But there is the compensating advantage, which is most obvious in haemorrhage, that the increased viscosity is due to the addition of essential elements to the blood, an advantage which, of course, does not exist in the case of gum. These remarks are not intended in any way to prejudge the question whether clinical experience or physiological experiments may not have demonstrated the superiority of solutions containing such substances as gum.

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THE EFFECT OF FEEDING PARS TUBERALIS AND PARS ANTERIOR PROPRIOR OF BOVINE PITUITARY GLANDS UPON THE EARLY DEVELOPMENT OF THE WHITE RAT

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INTRODUCTION

The pituitary body (hypophysis) is usually described as made up of two component parts, the anterior and posterior lobes (1).

Under the general term posterior lobe are included the pars nervosa, the pars intermedia and the neural stalk. Functionally these structures are very closely related. Extracts of each of them are capable of producing similar characteristic immediate effects when injected into the blood stream. It is believed that the active principles of the posterior lobe are elaborated in the pars intermedia and traverse the pars nervosa and neural stalk to the third ventricle, thus reaching the cerebrospinal fluid (2). The pars intermedia is histologically distinct from the pars nervosa and neural stalk, and traces its embryological origin to an entirely separate anlage. It is, however, very closely joined to the pars nervosa and is an essential part of the posterior lobe.

Injection of extracts prepared from the anterior lobe produce none of the effects characteristic of posterior lobe extracts. Long-continued feeding with the anterior lobe tissue, however, induces certain alterations in development, while the use in a similar way of posterior lobe preparations gives negative results.

Robertson in 1916 published a report in which he states that when fed to growing mice, anterior lobe produced a definite retardation of growth in the period immediately following puberty, this retardation being succeeded by a marked stimulation of growth (5). Goetsch, working with a much smaller number of white rats, stated that anterior lobe induced a uniform increase of growth-rate, denying the period of retardation (6). Later, Robertson and Delprat reported that anterior lobe feedings definitely increased the prepubertal growth rate of white mice.

The anterior lobe is also known to exert a certain control over the development and proper functioning of the reproductive system. Clinically, pituitary disease is always associated with more or less sexual abnormality, both anatomical and functional (9). Partial extirpation of the anterior lobe in experimental animals is followed by marked hypoplasia of the genitalia (3). Goetsch noted a marked hypertrophy of the genitalia in his pituitary-fed animals (6), while Robertson noted an increase in pugnacity and general virility in his pituitary-fed males (5).

Recently it has been shown that in addition to the structures mentioned above, the hypophysis contains a third epithelial lobe, the pars tuberalis, closely investing the neural stalk (10). The question arises whether this newly described structure is a part of the posterior or of the anterior lobe, or whether it possesses an entirely separate function. From its structural relations it might at first be considered a part of the posterior lobe complex for it is apparently continuous with the pars intermedia which has been proven to be a part of the posterior lobe mechanism. Atwell (11) has shown, however, that, in the rabbit at least, the pars tuberalis is embryologically quite distinct from the posterior lobe elements, arising as two lateral thickenings of the anterior lobe anlage and only later surrounding the developing neural stalk.

In a recent paper Atwell and Marinus (12) have compared the blood pressure and oxytocic reactions of pars tuberalis extracts with the reactions of pars intermedia, neural stalk and pars nervosa extracts. The reactions elicited by pars tuberalis extracts were so small that they were evidently due to unavoidable contamination of the extracts with active posterior lobe constituents, and not to any inherent activity on the part of pars tuberalis. It was concluded by the authors that the pars tuberalis is not a functional part of the posterior lobe.

Owing to its close anatomical relationship to the anterior lobe it was suspected that the pars tuberalis might be included in the ordinary anterior lobe preparations. Investigations showed that such was the case. As the anterior lobe is rolled from its capsule a small bit of the pars tuberalis is usually left attached to the pars anterior proprior. This fact opens the possibility that some of the effects of the anterior lobe feeding may be due to the inclusion of small portions of the pars tuberalis in the preparation. In the present study, white rats have been fed upon carefully separated pars tuberalis and pars anterior proprior, in an attempt to answer this question.

MATERIAL AND METHODS

In this experiment albino rats were fed portions of the pituitary gland, and carefully observed for any changes in growth rate and sexual development. The rats used were secured at the age of two to three weeks (shortly after weaning). They were immediately weighed and grouped according to weight and sex. Three separate series of experiments were

TABLE 1

Experiment 1. Gain in body weight (in grams)

SEX		AFTER 6½ WEEKS OF FEEDING			AFTER 12 WEEKS OF FEEDING		
		Group I. Anterior lobe	Group II. Pars tuberalis	Group III. Meat	Group I. Anterior lobe	Group II. Pars tuberalis	Group III. Meat.
Very young females	Number in group	6	2	4			
	Average final weight	72.6	68.0	73.1	Not weighed because of pregnancies		
	Average initial weight	16.0	16.7	17.0			
	Average gain	*56.6*	*51.3*	*56.1*			
Older females	Number in group	4	3	3			
	Average final weight	98.2	83.1	97.8	Not weighed because of pregnancies		
	Average initial weight	36.4	35.3	38.8			
	Average gain	*61.8*	*47.8*	*59.0*			
Very young males	Number in group		4	2		4	2
	Average final weight		74.7	84.2		90.5	97.0
	Average initial weight		18.2	16.7		18.2	16.7
	Average gain		*56.5*	*67.5*		*72.7*	*80.3*
Older males	Number in group	3	3	3	3	3	3
	Average final weight	113.6	107.1	113.5	130.3	121.3	134.3
	Average initial weight	38.3	38.6	46.0	38.3	38.6	46.0
	Average gain	*75.3*	*68.5*	*67.5*	*92.0*	*82.7*	*88.3*

conducted, one group comprising fifty-three rats, the second, thirty-seven, and the third, ten. The third series contained a single litter born at this laboratory.

During the entire experiment the rats were kept on a standard diet consisting of water and cracked mixed grains in abundance, stale (but not mouldy bread) once a week, and a small quantity of fresh milk daily. The rats were kept in metal cages, without litter except for the

grain they scattered about. The cages were cleaned weekly, using a coal-tar disinfectant. Individual rats were distinguished by spotting with stains—methylene blue, acid fuchsin and picric acid proving the most satisfactory. Each rat was weighed twice weekly.

In addition to the standard diet the rats were daily given the experimental foods. A small portion of the fresh gland was presented to the rat in forceps, whereupon it was invariably seized and swallowed. At

TABLE 2

Experiment 2. Gain in body weight (in grams)

SEX		AFTER 5½ WEEKS OF FEEDING			AFTER 11 WEEKS OF FEEDING		
		Group I. Anterior lobe.	Group II. Pars tuberalis	Group III. Meat.	Group I. Anterior lobe	Group II. Pars tuberalis	Group III. Meat
Very young females	Number in group	3	3	4			
	Average final weight	61.3	61.3	63.2	Not weighed because of pregnancies		
	Average initial weight	22.5	27.5	26.1			
	Average gain	*38.8*	*33.8*	*37.1*			
Older females	Number in group	5	4	6			
	Average final weight	94.9	91.2	94.3	Not weighed because of pregnancies		
	Average initial weight	44.8	47.1	45.5			
	Average gain	*50.1*	*44.1*	*48.8*			
Very young males	Number in group	7	5	5	7	5	5
	Average final weight	76.4	62.9	73.9	116.4	94.0	109.0
	Average initial weight	29.5	30.1	30.0	29.5	30.1	30.0
	Average gain	*46.9*	*32.8*	*43.9*	*86.9*	*64.1*	*79.0*
Older males	Number in group	4	4	3	4	4	3
	Average final weight	109.4	90.0	101.8	148.5	119.5	138.5
	Average initial weight	46.9	44.0	45.3	46.9	44.0	45.3
	Average gain	62.5	46.0	56.5	101.6	75.5	93.2

each feeding it was definitely ascertained that the portion allotted was completely devoured. The glands used were taken indiscriminately from steer, cow and heifer heads. As soon as possible after the animal was slaughtered the head was split in the sagittal plane and the pituitary gland dissected out. The glands were immediately wrapped in oiled paper and packed in ice. In every case the glands were dissected into their component parts and fed to the rats within six hours after the

TABLE 3

Experiment 3. Gain in body weight (in grams). After 6½ weeks of feeding

	GROUP I. ANTERIOR LOBE			GROUP II. PARS TUBERALIS			GROUP III. MEAT		
	Initial weight	Final weight	Gain	Initial weight	Final weight	Gain	Initial weight	Final weight	Gain
1. Female	32.0	105.5	73.5	32.0	80.5	48.5	32.5	96.0	63.5
2. Female	35.0	118.5	83.5	34.0	88.5	54.5	33.0	94.0	61.0
3. Male	36.5	129.5	93.0	32.0	100.0	78.0	37.0	112.5	75.5
4. Male				33.5	87.5	74.0			
Total	103.5	353.5	250.0	131.5	350.5	255.0	102.5	302.5	200.0
Average	34.5	117.8	*83.3*	32.9	89.1	*63.7*	34.1	100.8	*66.6*
Relative gain			125.0			95.0			100.0
Normal valued at 100				(cf. table 5)					

TABLE 4

Experiment 3. Body lengths in centimeters after 6½ weeks of feeding

	GROUP I. ANTERIOR LOBE	GROUP IV. PARS TUBERALIS	GROUP VII. MEAT
1. Female	15.2	13.8	13.6
2. Female	16.2	14.8	13.9
3. Male	16.3	14.6	15.2
4. Male		13.6	
Total	47.7	56.7	42.7
Average	15.9	14.2	14.2

TABLE 5

Experiment 3. Weights of genital organs in milligrams

	GROUP I. ANTERIOR LOBE		GROUP II. PARS TUBERALIS		GROUP III. MEAT	
	Male	Female	Male	Female	Male	Female
1		160		120		115
2		220		100		140
3	1530		885		910	
4			865			
Average	1530	190	875	110	910	135
Relative variation	168		96		100	
Normal valued at 100		140		81		100

death of the animal. During this time there was no actual contact between the ice or ice water and the glands. These precautions were taken in order to make certain that the material should lose none of its activity by autolysis, by decomposition or by solution of its constituents in the ice water.

The partes tuberales were dissected out as described by Atwell and Marinus. The material available was then divided into equal portions sufficient to supply all the rats to be fed. Each rat was given a quantity of pars tuberalis which varied from day to day but which approximated one-fourth of an entire pars tuberalis.

TABLE 6

Experiment 2. Birth of first litter and weight of mother at conception

	GROUP I. ANTERIOR LOBE		GROUP II. PARS TUBERALIS		GROUP III. MEAT	
	Litters	Weight	Litters	Weight	Litters	Weight
7th week of feeding	1	101.0				
8th week	2	94.0				
		90.0				
9th week						
10th week	1	107.5				
11th week	2	91.5				
		121.5			1	107.0
12th week			1	122.0	2	94.0
						89.5
13th week			2	94.5	2	127.0
			(2 preg.)	85.5	(3 preg.)	121.5
Average weight		*100.9*		*100.6*		*107.8*

The anterior lobes were secured by splitting the glands in the midsagittal plane and rolling the entire anterior lobe out of its capsule, leaving the posterior lobe attached to the capsule and dura mater. The portion of the anterior lobe at the point of origin of the pars tuberalis was snipped off, making certain that no pars tuberalis fluids or tissues were included with the anterior lobe as fed. Each rat was given a portion equal to about one-sixth of the entire lobe (approximately 250 mgm.). As a control certain of the rats were fed beef muscle. A portion of the muscle was freed from all loose fat and was then divided into portions approximately equal in weight to the portions of anterior lobe.

All the rats in series 3 (see above) were killed with ether after six and one-half weeks of feeding. The body weights and lengths were recorded. The entire female genital tract was dissected out down to the trigone and preserved intact. The sex organs were then fixed in Bouin's fluid and dehydrated in alcohols. When in 80 per cent alcohol each testis (together with the corresponding epididymis) was weighed separately. The female genital tract was weighed as a whole. The tissues were completely dehydrated, cleared with xylol, imbedded in paraffin and cut 5 micra thick. The sections were stained with Heidenhain's iron haematoxylin and eosin.

DISCUSSION

Puberty occurs in the white rat from sixty to ninety days after birth (13). The rats of experiments 1 and 2 were then barely approaching the normal time of puberty after six and one-half and five and one-half weeks of feeding. This stage corresponds roughly to the end of the second or beginning of the third growth cycle described by Robertson for white mice. Comparison of the weights at this stage (tables 1 and 2) shows a small but well defined increase in weight in the anterior-lobe-fed groups over all other groups. This is in agreement with Robertson and Delprat who conclude that anterior lobe feeding stimulates growth during the second development cycle.

The rats of experiment 3 were born in the same litter and allow more exact comparisons. Here also the anterior-lobe-fed surpassed the other animals at the age of nine weeks (after six and one-half weeks of feeding) (table 3). The difference is even more marked in this experiment owing to the greater uniformity of the animals.

After twelve weeks of feeding all the females had given birth to at least one litter or were in some stage of pregnancy. The rats had, therefore, all passed puberty. At this stage, also, the anterior-lobe-fed males were heavier than any of the others, the difference being greater than at the age of puberty. This observation is contrary to Robertson's conclusions that after puberty the administration of anterior lobe to white mice produces a preliminary retardation of growth. The seeming inconsistency may be due to a possible difference in the relative lengths of the growth cycles of white mice and white rats.

The nose-to-anus lengths recorded in table 4 show that the anterior-lobe-fed rats were considerably larger than either the controls or the pars-tuberalis-fed rats. The increased weights noted in the case of the

anterior-lobe-fed rats are, then, due to an increased skeletal growth. This conclusion is in keeping with the increased skeletal growth noted in cases of pathologic hyperpituitarism.

While the anterior-lobe-fed rats were growing faster than the controls, the pars-tuberalis-fed animals were losing ground. In all three experiments the pars-tuberalis-fed rats were markedly smaller than the controls. This was even more constant and more marked than the overgrowth of the anterior-lobe-fed animals. It should be remarked, however, that the pars-tuberalis-fed rats were not strictly controlled. The rats of the other groups received daily a quantity of muscle or glandular tissue weighing approximately 250 mgm. while the tuberalis-fed rats were given only a minute portion of tissue weighing but 5 to 10 mgm.

It is evident that the accelerated growth reported by previous observers as following anterior-lobe-feeding has not been due to any admixture of pars tuberalis substance, inasmuch as the same effects are produced by anterior lobe material known to be free from other parts of the gland, and inasmuch as no overgrowth is induced by feeding pars tuberalis alone. The apparent retardation of growth must be further studied before it is to be definitely ascribed to pars tuberalis feeding.

In addition to its action on the process of growth, the anterior lobe is known to control, to some extent at least, the development of the reproductive system. The effects of anterior lobe feedings on the sexual organs may be evaluated by *a*, study of the organs themselves, or *b*, date of birth of the first litter. Both methods have been used in this study.

The testes of the anterior-lobe-fed male (experiment 3) were markedly larger than those of the other males, weighing nearly twice as much as the controls. Upon section it was found, as reported by Goetsch, that all of the tissues in the testicle are involved in the hyperplasia but that the increase in size and weight is chiefly attributable to changes in the seminiferous tubules. The female organs were also better developed although the gross difference was not so marked.

The genital organs of the pars-tuberalis-fed rats could not, however, be distinguished from those of the controls. The slightly greater weight of the control organs is within the limits of individual variation and is explained as concomitant to the larger size of the control animals. Microscopical examination of both testes and ovaries revealed no difference between those of the pars-tuberalis-fed and control rats.

In normal healthy rats the first copulation occurs shortly after the sex glands become histologically mature. Thus the date of birth of the first litter is a good criterion of the relative maturity of two groups of animals. During the ninth week of feeding it became evident that several of the anterior-lobe-fed animals were pregnant, while none of the controls were showing signs of pregnancy. This observation was borne out by the fact that three anterior-lobe-fed females gave birth to the first litters two weeks before any of the control litters were born. The females evidencing this sexual precocity were not larger than the slower controls at the calculated date of impregnation, i.e., the sexual development had been more rapid than the somatic.

The pars-tuberalis-fed and control females dropped their litters during the same period of time, averaging more than two weeks after the anterior-lobe-fed females. In keeping with the microscopical evidence afforded by experiment 3, the births in this experiment show that the pars-tuberalis-feedings do not alter the sexual development or function of the white rat.

SUMMARY AND CONCLUSIONS

One hundred young rats were separated into three groups. The first group was fed upon pars anterior proprior of the pituitary gland, the second upon pars tuberalis, and the third or control group upon beef muscle. During twelve weeks of feeding the rats of the first group exhibited increased growth rate accompanied by a more rapid development of the reproductive system, evidenced by gross and microscopical hypertrophy of the organs and by the earlier birth of young. In the second (pars tuberalis fed) group there was no change in the sexual development as compared with that of the control group. The growth rate was slightly slower in the second group, owing perhaps to the smaller amount of meat fed.

This study has not shown that any of the functions ascribed to the anterior lobe as a whole are due to the pars tuberalis.

I wish to express my indebtedness to Dr. Wayne J. Atwell for his valuable assistance in the preparation of this paper.

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THE RELATION OF HYPOPHYSIS TO GLYCOGENOLYSIS

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In a previous report it was shown by the authors (1) that stimulation of the hypophysis produced a hyperglycemia, which did not occur if the stimulus was applied contiguous to but not on the gland, and that this rise in sugar was absent in animals whose splanchnic nerves had been previously sectioned. The present study was undertaken to determine the significance of this apparent nervous relation of the hypophysis to the glycogenolytic process.

The same general plan of experimentation previously adopted was followed. The blood sugar however was estimated by the Benedict method as modified by Meyers and Bailey (2) and by Benedict (3) rather than by the Bertrand (4) titration after the removal of proteins in acid sodium sulphate solutions.

Stimulation of gland. It was soon observed that under etherization alone the colorimetric methods gave a continuous persistent rise in glycemia despite the use of precautions to rule out all influences other than ether. A study of this mechanism was undertaken and has been recently reported by one of us (5).

Stimulation of the hypophysis gave in some of the animals a glycemic level which was no higher than that attained by ether stimulation alone. In other cases the level was distinctly above that ever obtained by simple etherization. Responsiveness of different animals to such experimental procedure shows rather wide individual variations, so that it is difficult to establish a normal. Consequently a quantitative value cannot always be assigned to the ether hyperglycemia. However, the character of the curves under the two experimental conditions differ. Ether shows a slow rise with a maximum attained in the interval between second and third hour. With constant ether administration the curve appears within these ranges to be a simple function of the time. Following the excitation of the hypophysis, whether it be done mechanically (as in

drilling the bone out over the gland or manipulating it in its removal), or electrically, there is an immediate step up in the blood sugar. The quantity of sugar so liberated may be greater than would appear under prolonged etherization but of this we have no conclusive proof. However, the evidence furnished on stimulating the gland in animals with denervated livers is strongly suggestive that an increase above the ether level occurs. Certainly it is clear that there is an immediate acceleration of the glycogenolytic process.

TABLE 1

Stimulation of hypophysis in normal animals

ANIMAL	BEFORE ETHER	AFTER				
		Ether	Drilling†	Stimulation	Rest	Time of experiment in minutes
*1	0.094	0.091	0.088	0.100	0.106	73
2	0.088	0.113	0.182	0.232	0.183	75
3		0.124	0.176	0.193	0.169	55
4		0.115	0.136	0.260	0.273	63
5		0.145	0.201	0.243		105
6	0.096	0.133	0.146	0.155	0.160	80

* Attempt had been made to section cord in this animal previously. Appetite good but he never regained complete health.

† Gland exposed by using a dental drill.

The results are the average of duplicate estimations, figured to the percentage of dextrose in the whole blood.

Doubly splanchnectomized animals. In the previous report it was shown that when the splanchnic nerves were cut the rise in sugar did not follow the hypophyseal stimulation. This finding was confirmed in two animals:

Dog 25. Splanchnics were sectioned 17 days previously; the blood sugar after relaxation under surgical anesthesia 0.128 per cent; 1 hour and 43 minutes later during which gland was exposed and stimulated, 0.093.

Dog 26. Splanchnics cut 7 days previously; after surgical anesthesia, 0.087; 45 minutes later during which the gland was exposed and stimulated, 0.091.

Section of nerve in hepatic pedicle. In the first two cases (animals 7 and 8) only the nerves about the hepatic artery were cut. In the others (9, 10, 11) all of the structures in the hepatic pedicle were severed. The coats of the artery vein and common bile duct were cauterized lightly with hot iron. This precaution, as had been shown by Macleod (6),

must be taken if one is to be certain of a complete separation of liver from its nervous connections.

Animals so operated have been shown previously (5) to give a lower hyperglycemic response to ether than normal animals. The last column of table 2 shows these ether control values reproduced from the former study. Table 2 indicates quite clearly that the blood of these animals attains a high grade of hyperglycemia following excitation of the hypophysis. This rise in comparison with the control experiments suggests further that stimulation of the gland probably not only initiates and accelerates the glycogenolysis, but also raises it to higher level than ether alone does under similar experimental conditions.

TABLE 2

Effect of stimulation of the hypophysis after section of nerves in the hepatic pedicle

ANIMAL	BEFORE ETHER	AFTER				
		Ether	Drilling	Stimulation	Rest	Control after 3 hours ether anesthesia
Hepatic artery denervated*						
7	0.101	0.137	0.208	0.216	0.234	
8		0.171	0.201	0.225	0.234	
Section of all nerves in pedicle†						
9	0.105	0.148	0.167	0.248	0.277	0.132
10	0.083	0.106	0.161	0.168	0.163	0.124
11	0.090	0.095	0.133	0.164		0.117

* Nerves removed from artery alone and coats cauterized.

† Entire pedicle sectioned except hepatic artery, portal vein and common bile duct. Coats of these were cauterized.

Transection of cord. The cord was transected by removing the second dorsal spine and severing it with a sharp-pointed probe. It was suggested in our former study that the alleged hormone, which Weed, Cushing and Jacobsen (7) believed they had liberated in the stimulation of the hypophysis and the superior cervical ganglion, might really exist and might find its site of action on the terminations of the pre-ganglionic splanchnic fibers which we had destroyed in our splanchnectomy.

In order to test out this hypothesis, the experiments on animals with transected cords were performed. Reference to table 3 shows that such animals were used in all stages of recovery (from four to thirty-six

days), so as to eliminate the vasomotor instability associated with spinal shock and that in none of these was there a suggestion of hyperglycemia.

TABLE 3

Stimulation of hypophysis in animal with cord transected at the level of second thoracic vertebra

ANIMAL	DAYS AFTER TRANSECTION	BEFORE ETHER	AFTER			
			Ether	Drilling	Stimulation	Rest
12	4	0.108	0.111	0.117	0.076	0.088
13*	6		0.112	0.060	0.076	0.082
14	16	0.077	0.093	0.077	0.071	0.063
15	21	0.102	0.091	0.098	0.089	0.073
16*	25	0.069	0.087	0.084	0.112	0.111
17	36	0.104	0.097		0.088	0.071

* Sugar estimated by sodium sulphate precipitation: Bertrand titration; others by colorimetric method.

TABLE 4

Glycemia following hypophysectomy

ANIMAL	AFTER ETHER	AFTER DRILLING	AFTER REMOVAL OF GLAND	
			Immediately	Hours expressed as exponents
Normal animal				
18	0.143	0.227	0.277	0.093^{3}; 0.055^{6}; 0.083^{24}; 0.062^{47}; 0.067^{100}
19	0.143	0.142	0.137	0.093^{3}; 0.099^{5}; 0.072^{23}; 0.062^{46}
20	0.143	0.273	0.311	0.1001^{3}; 0.092^{23}
21	0.153	0.146	0.153	0.107^{4}; 0.092^{22}; 0.083^{46}; 0.103^{72}; 0.103^{44}
Complete denervation of hepatic pedicle				
22	0.143	0.168	0.330	0.134^{5}; 0.090^{23}
23	0.106	0.161	0.156	0.136^{4}; 0.090^{27}
24	0.090	0.134	0.167	0.085^{4}; 0.069^{23}; 0.089^{29}
Splanchnic nerves on both sides sectioned				
25	0.128	0.124	0.102	0.095^{5}; 0.094^{24}

Hypophysectomy. Cushing and associates (8) have reported that in dogs a transitory glycosuria follows hypophysectomy, manipulation of gland or compression of stalk with a metal clip. The literature has been

critically reviewed by Goetsch (9) but these workers are apparently the only ones who have investigated the relation of hypophysis to carbohydrate metabolism using the methods of glandular removal and electrical stimulation. We desired to know the duration and persistence of this hyperglycemia, so glands were removed in several animals as shown in table 4. Examination of the data shows that within three to five hours after the removal, the sugar level is normal and remains so until death.

In animal 19 there appeared to be some evidence of hypoglycemia but this we attributed to his moribund condition. No attempt was made to determine the completeness of the removal by microscopic section, since the animals died with all the evidences of insufficiency within the usual time limits.

DISCUSSION

The data presented demonstrate clearly that if the stimulus be prevented from reaching the level of the adrenals no hyperglycemia results from stimulation of the hypophysis. A hormone[1] if liberated by the stimulation does not act on any of the peripheral mechanism of glycogenolysis. For section of the cord leaves the splanchnic nerves (both pre- and post-ganglionic fibers), all their terminations in the adrenals and liver intact, and yet, following this, the experiment does not give a hyperglycemia. Such a hormone would have to find its site of action on some central cranial structure.

If the pathway be assumed to be a nervous one, then section of splanchnics removes the block a little further peripheralward, a procedure which prevents the hyperglycemia. Section in hepatic pedicle beyond the adrenals does not interfere with the rise in blood sugar. These findings are all analogous to those of Macleod (10) on stimulating splanchnic nerves, and Keeton and Ross (5) in their study of ether hyperglycemia. It appears then that excitation of the hypophysis has originated a stimulus which is directed peripheralward by nervous connections and falls upon the splanchnic-adrenal-hepatic mechanism controlling the ultimate partition of glycogen.

The transitory nature of the hyperglycemia following the removal of the hypophysis coupled with the maintenance of a fairly normal sugar level until death we believe points to the fact that the rôle played by the hypophysis in carbohydrate metabolism deals not with the

[1] Under the term "hormone" is included any chemical product formed in one organ which may stimulate any other organ to activity.

process of glycogenolysis, but with some other phase. Indeed the well established alteration of sugar tolerance in cases of acromegaly and pituitary tumors (11) as well as the studies of Cushing in carbohydrate tolerance after removal of the posterior lobes indicate that future investigation should be directed toward the influence of the hypophysis on the utilization of the sugar by the organism.

CONCLUSIONS

1. Stimulation of hypophysis in dogs causes hyperglycemia independently of the ether used in the anesthesia.

2. This hyperglycemia is absent after transection of cord at level of second thoracic vertebra and after section of the splanchnics. It persists after section of the nerves in the hepatic pedicle.

3. Following hypophysectomy a transitory hyperglycemia occurs, and persists three to five hours. After this the sugar level remains normal until death.

4. If a hormone is liberated by stimulation of the gland, it must have a central action.

5. The view is favored that the pathway is a nervous one mediated through the splanchnic nerves to their terminations in the adrenals and liver.

6. The physiological rôle played by the hypophysis in carbohydrate metabolism does not deal with transformation of glycogen into sugar, but more probably with the utilization of the sugar by the organism.

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THE GASTRIC RESPONSE TO FOODS[1]

VI. Digestion in the Normal Human Stomach of Eggs Prepared in Different Ways

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Eggs like meats are particularly rich in protein. Hence their digestion is carred out to a considerable extent in the stomach and is of more than usual interest not only from the standpoint of normal nutrition but also from that of clinical dietetics, inasmuch as eggs in the raw state or prepared in different ways are frequently used to replace meat in the diet of invalids.

Beaumont (1) found that raw egg white left the stomach of his subject (Alexis St. Martin) very quickly, in fact sooner than any other food investigated. Pavlov (2) noted in dogs that such egg white produced no stimulation of gastric secretion. Bateman (3) found the utilization of raw egg white to be poor, particularly when large amounts were fed to dogs, and that this indigestibility was due to the ovalbumin which the egg white contains. Penzoldt (4) studied the evacuation times of eggs in the human stomach, using a large stomach tube, and obtained as the result of five experiments the values given in table 1.

Jaworski and Gluzenski (5) reported that it required one and a quarter hours for the white of one egg, finely chopped and taken with two and one-half ounces of water, to leave the stomach. Water has, however, in itself a stimulatory effect upon secretion (6).

The present study was made to determine the response of the normal human stomach to eggs prepared in different ways. The fractional method of gastric analysis was employed to follow the course of digestion. The experimental conditions and the methods employed were in general those used in the earlier work carried out in this laboratory upon meats

[1] The expenses of this investigation were defrayed by a fund furnished by Mrs. M. H. Henderson, the Curtis Publishing Company and Dr. L. M. Halsey.

(7). The subjects were normal medical students who came to the laboratory at 8:00 a.m. without breakfast. Residuums were not removed. The number of eggs given to each man was two except where otherwise specified. The subjects were permitted to eat these in their ordinary manner. The time required was about ten minutes with not more than two or three minutes variation. Most of the subjects of these tests swallowed the tube without assistance and showed no signs of discomfort. They remained seated most of the time and at rest, i.e., talking, reading and copying notes. Samples were withdrawn at 15 minute intervals and kept in ice water for the short period preceding analysis. Lavage was used in every case to determine conclusively whether the stomach was empty or not. In practically every case the results of lavage were confirmatory, showing that the stomach can be completely emptied by means of the tube used. In a few cases it was necessary to discontinue the experiment after a period of hours before digestion was complete.

TABLE 1

Evacuation times of eggs from the stomach (Penzoldt)

PREPARATION	EVACUATION TIME
	hours
2 lightly boiled eggs	1¾
2 raw eggs	2¼
2 poached eggs, 5 grams butter	2½
2 hard boiled eggs	3
2 eggs in omelet	3

In such cases the stomach was emptied and the volume of contents determined. All experiments were carried to completion except where otherwise indicated on the charts. Subjects showing any indication of pathological response were rejected. Free acidities are distinguished in charts from total acidities by using the prime mark as A′.

Among the egg preparations tested were raw white, raw yolk, raw whole eggs (strained and unstrained), soft boiled, hard boiled, scrambled (with and without excess of fat), fried (with and without excess of fat), plain omelet, Spanish omelet, deviled eggs, poached eggs, pickled eggs, shirred eggs and soft cooked eggs. Frozen eggs and cold storage eggs were compared with fresh eggs. Duck and turkey eggs were also used as well as the Chinese preserved duck egg called pidan. Eggs with milk, French toast or eggs with bread, scrambled eggs with frizzled beef, and bacon and eggs were food combinations tested. A few tests were made on raw eggs with water and egg albumin with orange juice.

TABLE 2

Response of the normal human stomach to eggs prepared in different ways

NUMBER	KIND OF PREPARATION	RAPID-TYPE INDIVIDUAL				SLOW-TYPE INDIVIDUAL			
		Evacuation time		Highest acidity*		Evacuation time		Highest acidity*	
			Average	Average			Average	Average	
	Hens' eggs								
1	Raw white	1:30		37					
2	Raw white	1:00		83					
3	Raw white	2:00	1:30	90	70				
3a	Raw white					2:15	2:15	6.0	6.0
4	Raw yolk	2:15	2:15	94	94				
5	Raw yolk					3:00		84	
6	Raw yolk					2:45			
7	Raw yolk					2:00	2:35	105	95
8	Raw eggs (whole)					2:15		107	
9	Raw eggs (whole)					3:30	2:55	43	75
10	Raw eggs (strained)	3:00		81					
11	Raw eggs (strained)	3:10							
12	Raw eggs (strained)	2:15		80					
13	Raw eggs (strained)	2:00	2:35	71	77				
14	Raw eggs (strained)					3:00	3:00	53	53
15	Soft boiled	2:00		94					
16	Soft boiled	1:45		106					
17	Soft boiled	2:30		68					
18	Soft boiled	2:00		63					
19	Soft boiled	1:45		94					
20	Soft boiled	2:45	2:00		85				
21	Soft boiled					2:45		122	
22	Soft boiled					2:50			
23	Soft boiled					3:00		79	
24	Soft boiled					2:00		48	
25	Soft boiled					4:05			
26	Soft boiled					3:30		58	
27	Soft boiled					3:00	3:00		77
28	Hard boiled	2:00		92					
29	Hard boiled	3:00		132					
30	Hard boiled	1:30		82					
30a	Hard boiled	2:30		101					
31	Hard boiled	2:15		70					
32	Hard boiled	1:45	2:10	93	95				
33	Hard boiled					3:15			
34	Hard boiled					3:25			
35	Hard boiled					3:30			

TABLE 2—*Continued*

NUMBER	KIND OF PREPARATION	RAPID-TYPE INDIVIDUAL				SLOW-TYPE INDIVIDUAL			
		Evacuation time		Highest acidity		Evacuation time		Highest acidity	
			Average	Average			Average	Average	
36	Hard boiled					3:00		107	
37	Hard boiled					3:30	3:20	97	102
38	Scrambled	2:30		86					
39	Scrambled	2:15	2:25	100	93				
40	Scrambled					2:45		81	
41	Scrambled					3:50			
42	Scrambled					3:15	3:15	94	85
43	Fried	2:00		106					
44	Fried	2:30	2:15		106				
45	Fried					2:00	2:00	108	108
46	Fried (excess fat)	2:45	2:45	91	91				
47	Fried (excess fat)					3:00	3:00	90	90
48	Omelet plain	2:15		83					
49	Omelet plain	2:30	2:25	80	82				
50	Omelet Spanish	2:30	2:30	100	100				
51	Deviled eggs	2:45	2:45	103	103				
52	Deviled eggs					3:40	3:40		
53	Poached eggs	1:30	1:30	117	117				
54	Poached eggs					3:30	3:30	61	61
55	Shirred eggs	2:00	2:00	95	95				
56	Shirred eggs					2:30	2:30	85	85
57	Pickled eggs					3:30		85	
58	Pickled eggs					3:30	3:30	65	75
59	Scrambled (excess fat)	2:30	2:30	122	122				
60	Scrambled (excess fat)					3:30	3:30	58	58
61	Soft cooked	1:45	1:45	52	52				
62	Soft cooked					3:15	3:15	63	63
	Duck eggs								
63	Soft boiled					3:15	3:15	73	73
64	Hard boiled					3:00	3:00		
	Turkey eggs								
65	Hard boiled	2:45	2:45	103	103				
66	Hard boiled					3:15	3:15	88	88
	Frozen hens' eggs								
67	Scrambled	2:00	2:00	76	76				
68	Scrambled					2:30	2:30	79	79
69	Sponge cake (frozen eggs)	2:30		84					
70	Sponge cake (frozen eggs)	2:15	2:20	40	62				
71	Sponge cake (frozen eggs)					3:00	3:00	14	14
72	Sponge cake (fresh eggs)	2:00	2:00	42	42				
73	Sponge cake (fresh eggs)					2:30	2:30	15	15

TABLE 2—*Continued*

NUMBER	KIND OF PREPARATION	RAPID-TYPE INDIVIDUAL				SLOW-TYPE INDIVIDUAL			
		Evacuation time		Highest acidity		Evacuation time		Highest acidity	
			Average	Average			Average	Average	
	Cold storage eggs								
74	Soft boiled	1:45	1:45	91	91				
75	Soft boiled					2:30		93	
76	Soft boiled					3:30	3:00	66	80
77	Hard boiled					3:15	3:15	99	99
78	Fried (excess fat)	2:45	2:45	124	124				
	Egg combinations								
79	Milk and eggs	3:00		96					
80	Milk and eggs	2:45	2:50	41	68				
81	French toast					3:30		99	
82	French toast					3:30	3:30	67	83
83	Bacon and eggs	2:15	2:15	97	97				
84	Bacon and eggs					3:30	3:30	83	83
85	Frizzled beef and scrambled eggs	2:30	2:30	67	67				
86	Frizzled beef and scrambled eggs					2:45	2:45	146	146
87	Chinese pidan eggs	3:15	3:15	99	99				
88	Chinese pidan eggs					4:45	4:45	30	30
89	Raw hens' eggs and water					3:15	3:15	67	67
90	Raw white and water					2:15	2:15	85	85
91	Raw white and orange juice	1:15	1:15	86	86				
92	Raw white and orange juice					2:15	2:15	109	109

* Acidities expressed as cubic centimeters of N/10 alkali required to neutralize 100 cc. of sample.

Evacuation times and highest total acidities are recorded in table 2 which also gives average responses for individuals of the rapid- and slow-emptying types. The necessity for this division of subjects into classes is clearly apparent from an examination of the charts for certain of these individuals. Compare, for example, subjects Se and Da (figs. 1, 10 and 20) showing average evacuation times of about two hours for eggs with subjects Me and Ka (figs. 7, 19, 14 and 16) whose gastric digestion was not usually completed in three hours. Similarly the grand average evacuation time for 44 experiments on subjects of the rapid type was 2 hours 15 minutes and for 48 experiments on the slower type, 3 hours and 5 minutes.

In order that comparisons of the digestion of eggs prepared in different ways might be made as direct as possible, several experiments were made

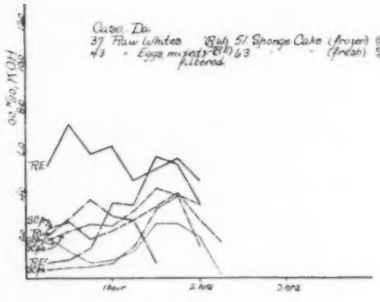

Fig. 1

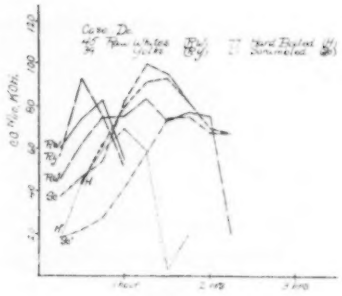

Fig. 2

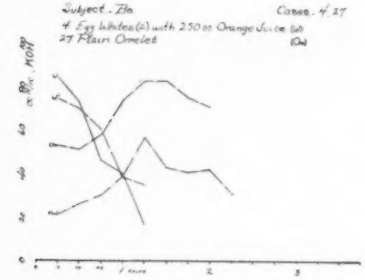

Fig. 3

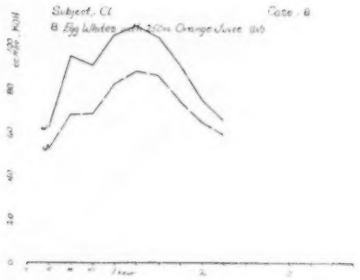

Fig. 4

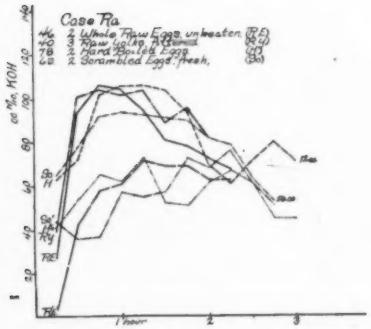

Fig. 5

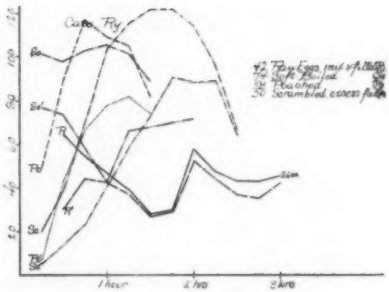

Fig. 6

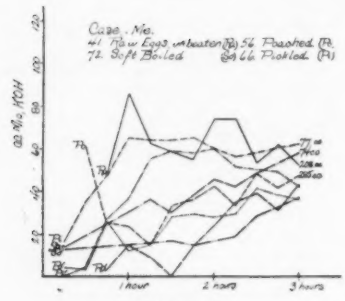

Fig. 7

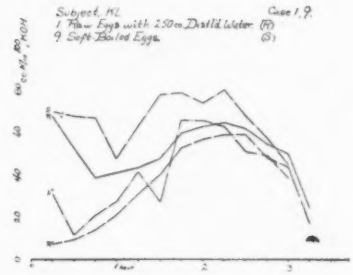

Fig. 8

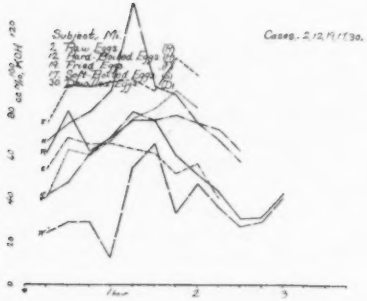

Fig. 9

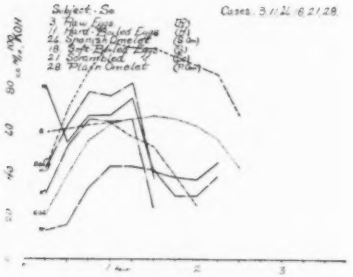

Fig. 10

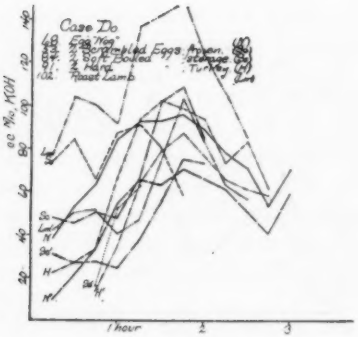

Fig. 11

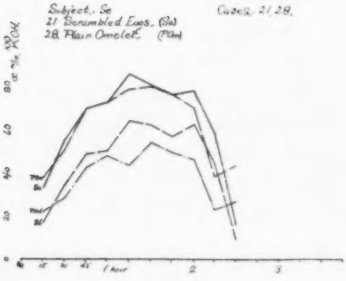

Fig. 12

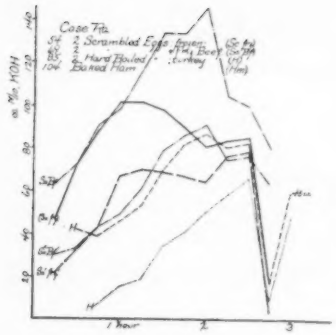

Fig. 13

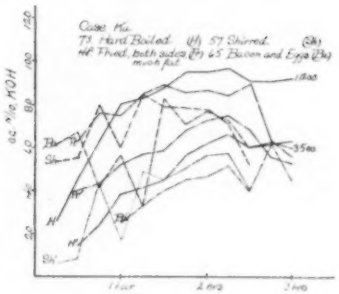

Fig. 14

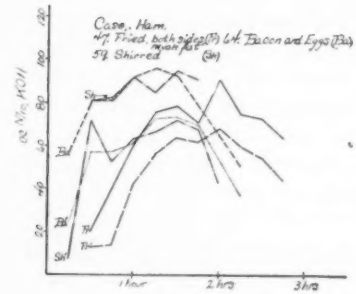

Fig. 15

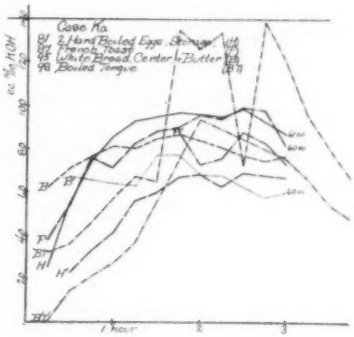

Fig. 16

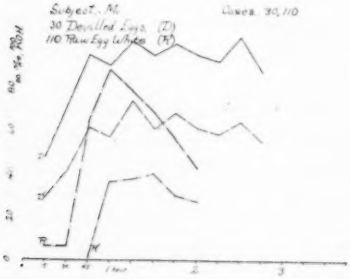

Fig. 17

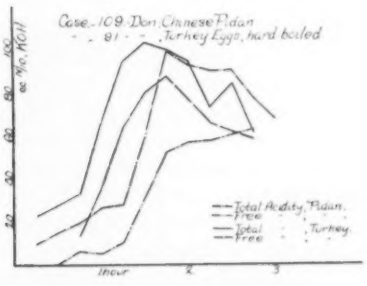

Fig. 18

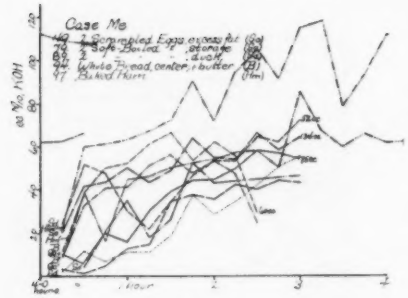

Fig. 19

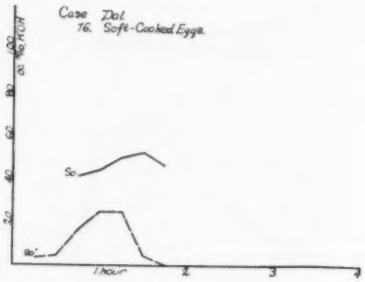

Fig. 20

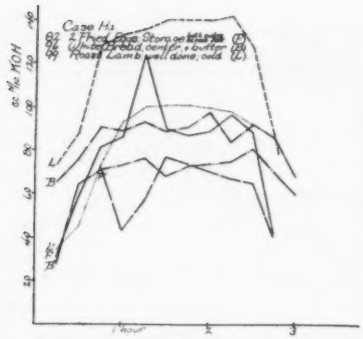

Fig. 21

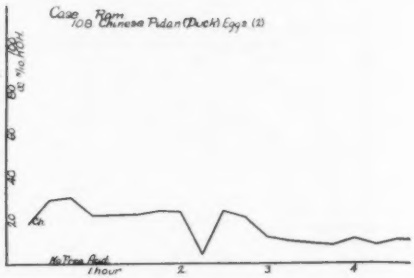

Fig. 22

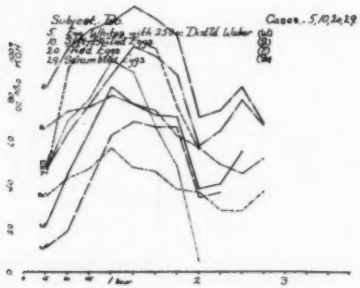

Fig. 23

upon most of the subjects used. About 75 of these experiments are charted in figures 1 to 23, which illustrate all of the most important facts brought out by this investigation.

DISCUSSION

The response of the stomach to raw eggs, raw white and raw yolk. In the experiments on raw egg white, the whites of three eggs were strained through cloth and given without any addition. This egg white left the stomach rather rapidly, i.e., in from 1 to 2¼ hours. (See table 2 and figs. 1, 2 and 17). In one case egg white showed hardly any stimulation of secretion, in another case the acidity was rather low, while two other cases showed a moderately high acidity. It appears that the stimulatory power of egg white is insufficient to cause any material secretion in individuals of a low acid type while subjects who respond readily to most food stimuli will show a response to egg white. Thus in cases Do (fig. 2) and Mi (fig. 17) a distinct stimulation of secretion by egg white occurred, although the secretion was not nearly as pronounced as with most cooked eggs. The finding of Pavlov (2) that egg white produced no stimulation of gastric secretion in dogs cannot be applied to man without qualification. The experiment on egg white charted in figure 17 was repeated the following day but instead of withdrawing samples at 15 minute intervals the entire gastric contents were aspirated after 45 minutes. Twenty cubic centimeters of liquid with a total acidity of 42 and a free acidity of 18 were withdrawn. Of the original 100 cc. of egg white given, not 10 per cent remained. The bulk of the egg white thus left very quickly but not without giving rise to a secretion of gastric juice which continued for an hour longer. Where 250 cc. of water were given with the egg white (exp. 90, fig. 23) gastric secretion was distinctly stimulated, not more so however than is usual with water alone (6). The addition of 200 cc. of orange juice (expts. 91 and 92 and figs. 3 and 4) led to a distinct acid secretion and rapid evacuation of the stomach. Experiment 92 (fig. 4) showing an evacuation time of 2¼ hours for albumin with orange juice should be compared with experiment 34 on the same subject showing an emptying time of 3¼ hours for hard boiled eggs and experiment 41 on scrambled eggs which required 3 hours and 50 minutes to leave the stomach. The use of egg albumin with orange juice in the diet of invalids would appear therefore to be supported from the standpoint of gastric digestion.

In the tests on egg yolk the yolks of three eggs were given after being strained through cheese cloth. Egg yolk shows a distinctly different response from the white. The time required for gastric digestion is notably longer. For instance subject Do (fig. 2) required 1 hour for egg white and 2¼ hours for the yolk. Higher total and free acidities were also developed by egg yolk. The high fat content of egg yolk (33 per cent) may well account for the delayed evacuation. In one case more or less pathological and therefore not included in the present series, a high total but no free acidity was developed. The acidity in this case must have been due to the action of regurgitated lipase on the fat of the yolk.

Two whole raw eggs were fed in certain cases. A number of tests were also made on such eggs after being mixed and strained through cloth. It might be expected that the response of the mixed white and yolk would be intermediate between that of yolk and white taken separately. It was found, however, that the response was much the same as that of yolk alone. In fact the average evacuation time for two whole eggs was greater than that for three yolks. Note the very similar response of egg and yolk on subject Ra (fig. 5). Note also the slower evacuation and higher acidity developed by whole raw egg as compared with egg white in the case of subject Da (fig. 1). Observe likewise the response of raw eggs as charted in figures 6, 7, 8, 9 and 10. The acidities developed (especially the free acidities) are lower than for most cooked eggs, but not markedly so.

Soft boiled and hard boiled eggs. The "soft boiled" eggs were placed in boiling water for 3½ minutes, the "hard boiled" eggs for 8 minutes. On the average the hard boiled eggs required a few minutes longer to digest than soft boiled eggs. Compare the two methods of cooking on subjects Mi (fig. 9), Se (fig. 10), Do (figs. 2 and 11), and note the similarity of response, in one case the evacuation times being the same, in another case the hard boiled having a slight advantage, while in the third the soft boiled left 15 minutes sooner. The acid response to hard boiled eggs was more marked in one case (fig. 9) but was the same as for soft boiled in the two other cases just mentioned.

Comparisons of raw eggs with boiled eggs are found in figures 5, 9 and 10. In cases 9 and 10, raw eggs took distinctly longer than either soft or hard boiled eggs. Apparently the stomachs of the rapid-emptying type handle the cooked eggs more readily than raw eggs.

Fried eggs and scrambled eggs. Scrambled eggs required on the average a little longer to digest than boiled eggs. Direct comparisons may be

obtained from figures 2 and 11, showing scrambled eggs to take ¼ to ½ hour longer to leave than either hard or soft boiled eggs; from figures 10 and 12, showing scrambled eggs to take 2½ hours as compared with 1½ and 2 hours for soft and hard boiled eggs respectively; from figures 5 and 13, which indicate that the boiled eggs had a very slight advantage; and from figure 23, showing scrambled and soft boiled eggs each to leave the stomach in 2¾ hours.

The effect of scrambling the eggs with a large excess of bacon fat was tested in two cases. In one case (fig. 19) (that of a slow-emptying stomach) the response was similar to that for soft boiled eggs, while in the other case, that of a rapid-emptying stomach, the scrambled eggs with excess fat remained an hour longer in the stomach.

Six tests were made on fried eggs particularly to determine the effect of frying on both sides with excess of bacon fat. This was done in view of the common opinion as to the indigestibility of greasy foods. Eggs fried in the ordinary way and not turned over left the stomach fully as soon as eggs cooked in any other manner. Compare experiments 21, 32, 40 and 45 on the same individual (fig. 23) showing evacuation times for fried eggs (2 hours), scrambled eggs (2¾ hours), for hard boiled (3¼ hours) and soft boiled (2¾ hours). Observe also figure 9, showing fried eggs to leave in 2 hours as compared with 2½ hours for hard boiled and 2¼ hours for soft boiled eggs. When excess of fat was used the emptying period was somewhat longer than for shirred eggs (fig. 15) but not as long as for hard boiled eggs (fig. 14). It appears that an excess of fat may delay the digestion of eggs appreciably in individuals of the rapid-emptying type but has little effect in the case of slowly evacuating stomachs. The common opinion that fried foods are less digestible was not borne out in the case of eggs. We have previously shown that fried meats are readily handled by the stomach (7).

Omelets, poached, shirred and soft cooked eggs. The digestion of plain and Spanish omelets was compared. Figures 10 and 12 show that the response of the stomach of this individual was identical in the two cases. The omelets required the same period of digestion as scrambled eggs and a longer period than hard or soft boiled eggs. That a plain omelet may remain in the stomach an hour longer than egg white with orange juice is indicated by figure 3.

Poached eggs were found to leave the stomach in the same time as soft boiled eggs in the case of a rapid-emptying individual (see fig. 6) and to require a little longer in the case of a slow-emptying individual (fig. 7). Shirred eggs appear to leave the stomach more rapidly than hard boiled

eggs or eggs fried with excess of fat (figs. 14, 15 and 16) and belong with the more readily digestible forms of eggs. Soft cooked eggs appear also to be digested in medium time (expts. 61 and 62 and figs. 1 and 20).

Pickled and deviled eggs. Eggs pickled in a vinegar extract of beets appeared to require about as long to digest as the hard boiled eggs from which they were derived (expts. 57 and 58 and fig. 7). Deviled eggs required $2\frac{3}{4}$ hours to leave the stomach as compared with $2\frac{1}{2}$ hours for hard boiled eggs and $2\frac{1}{4}$ for soft boiled eggs (figs. 9 and 17).

Duck and turkey eggs, Chinese preserved eggs. Soft and hard boiled duck eggs and hard boiled turkey eggs were compared with hens' eggs prepared in the same ways. Two eggs were given to each subject. A comparison of experiments 26 and 63 (figs. 7 and 19) and experiments 36 and 64 show that duck eggs were handled by the stomach in about the same time as hens' eggs. This was perhaps to be expected from the similarity in composition of ducks' and hens' egg.

Hard boiled turkey eggs were compared with hard boiled hens' eggs in two cases. In the completed experiments (figs. 2 and 11) the subject Do required $2\frac{3}{4}$ hours for the turkey eggs as compared with $1\frac{3}{4}$ hours for hens' eggs prepared in the same way. The difference is probably due to the larger size of the turkey eggs, whose contents weighed 77 grams each.

The Chinese preserved egg called "pidan" was tried out in two cases. These eggs have dark greenish yolks and yellow brown "whites" of a firm gelatinous consistency and possess distinct odors of ammonia and hydrogen sulphide. The period of retention of these eggs in the stomach we believe to have been influenced by their unappetizing character and in one case at least (fig. 18) to a prejudice against them. In one case (fig. 18) a single egg only was given. This remained in the stomach for three hours as compared with $1\frac{3}{4}$ hours for hard boiled hens' eggs (fig. 2). In the other case (figs. 13 and 22) two "pidan" eggs were given and remained in the stomach $4\frac{3}{4}$ hours as compared with 3 hours for hard boiled hens' eggs. The composition of these eggs has been studied by Blunt and Wang who have also carried out tests on the digestibility of these eggs in vitro (8). We desire to thank them for supplying us with the Chinese eggs used in our tests.

Cold storage and frozen eggs. An attempt was made to determine whether the keeping of eggs in cold storage for eight months had any effect upon their gastric digestibility when cooked in different ways. When the cold storage eggs were fried and compared with fresh eggs fried in the same way (figs. 15 and 21), it was found that both remained

in the stomach the same length of time and developed similar acidities. Another individual (figs. 2 and 11) evacuated hard boiled fresh eggs and soft boiled storage eggs in the same period ($1\frac{3}{4}$ hours) and the highest acidity in each case was about 90. No differences in the responses of the stomach to fresh and cold storage eggs were demonstrated.

The commercial frozen egg mixture was given to our subjects in the form of scrambled eggs and also made up into sponge cakes inasmuch as such eggs are mainly used in baking. Our two subjects who were given scrambled eggs both showed evacuation times a few minutes shorter for preparations made from frozen eggs than for the fresh egg preparations. The acidities developed were also practically identical (figs. 2, 11, 5 and 13).

Sponge cakes into which fresh and frozen eggs had been incorporated were likewise compared on two subjects, 100 gram portions being used. One of these comparisons is charted in figure 1. It shows a few minutes more rapid emptying where the fresh eggs were used but this difference is within the limit of experimental error. No appreciable differences in the action of the stomach on the two cakes could therefore be demonstrated nor does there seem to be any objection to the use of such eggs when canned under proper conditions.

Eggs used in combination with other foods. Several popular combinations of eggs with other foods were fed to certain of our subjects and all were found to be readily taken care of by the stomach. The combinations tested were: *a*, milk and eggs; *b*, bacon and eggs; *c*, French toast; *d*, frizzled beef with scrambled eggs.

The egg-milk combination used was an egg-nog consisting of 1 egg, $\frac{3}{4}$ cup milk, $\frac{3}{4}$ tablespoon sugar, $\frac{1}{4}$ teaspoonful vanilla extract and a sprinkle of salt and nutmeg. This combination took a little longer than eggs alone in the case of one man of a high acid, rapid-emptying type (fig. 11) but hardly as long in the case of a low acid individual (expt. 80).

The addition of eggs to milk distinctly alters the curdling effect of the gastric juice on such milk, the albumin preventing the formation of hard massive curds and giving rise to a light, flocculent precipitation of the casein.

The French toast used was made from 85 grams of bread with 2 eggs and fried in bacon fat. This toast required a little longer to digest than bread and butter alone but no longer than 2 hard boiled eggs (figs. 14 and 16) and must from this standpoint be considered as a satisfactory food.

Two eggs with bacon required in one case (fig. 14) only a little longer than fried eggs alone, while in another case (fig. 15) the bacon and eggs

left half an hour sooner. Individuals vary in their response to fatty foods but it appears that bacon and eggs are handled at least as readily as other foods high in fat and protein.

The frizzled beef and scrambled egg preparations used were made from 2 eggs with 25 grams chipped beef and a tablespoonful each of cream and butter. This chipped beef preparation took no longer to digest than scrambled eggs alone and gave rise to a more pronounced acid secretion (figs. 5 and 13). Meat-egg preparations would therefore appear to possess certain advantages over eggs alone.

Eggs are distinguished from meats by the lower acidities which they provoke and by their more rapid evacuation. As examples of this note charts 15, 16 and 21, illustrating the digestion of roast lamb and boiled tongue as compared with eggs. The results presented in this paper should also be compared with our findings on meats as presented in preceding papers (7). The average of the highest total acidities for all egg tests was about 80, and for free acidities 60. Meats on the other hand showed an average total acidity of 125. The average evacuation time for subjects of the rapid type on beef and beef products was 2 hours and 35 minutes as compared with 2 hours and 15 minutes for eggs and egg preparations. Subjects of the slow-emptying type required on the average 3 hours and 25 minutes for beef as compared with 3 hours and 5 minutes for eggs. Pork required a longer time than beef.

SUMMARY AND CONCLUSIONS

A series of over 90 experiments on 18 different subjects was carried out to determine the response of the normal human stomach to eggs prepared in various ways. Two eggs were used as the experimental meal except where otherwise specified. The fractional method of gastric analysis was employed. The evacuation times and highest acidities have been tabulated and curves plotted to show the comparative responses of certain subjects to different egg preparations.

The subjects were classified as belonging either to the rapid- or to the slow-emptying types. The average evacuation time for all egg preparations was for the first class 2 hours and 15 minutes and for the second class 3 hours and 5 minutes.

Eggs give rise to less stimulation of gastric secretion than meats and leave the stomach sooner. Beef, for example, showed an average emptying time of 2 hours and 35 minutes for the rapid- and 3 hours and 25 minutes for the slow-emptying type. The average of the highest

acidities developed in egg experiments was 80 as compared with 120 for beef. In general eggs show high combined acidities throughout the early period of digestion.

Raw egg white left the stomach much more rapidly than any other form of egg preparation. A moderate secretion of gastric juice was induced in subjects of a high acid type, but this did not become apparent until most of the egg white had left the stomach without being acted upon by the gastric juice. Egg white with 200 cc. of distilled water produced a more marked stimulation of acid secretion. Egg white with 200 cc. of orange juice led to a distinct gastric secretion and a rapid evacuation of the stomach.

Raw egg yolk required much longer to leave the stomach than egg white and higher acidities were developed. Whole raw eggs resemble egg yolk in their response whether unmixed or strained through cloth, showing the same delayed evacuation and greater acid stimulation as compared with egg white. Raw eggs produced somewhat less stimulation of acid secretion than boiled eggs and remained longer in the stomach.

Hard boiled eggs required on the average a few minutes longer for gastric digestion than soft boiled eggs but the acid response was similar in the two cases.

Scrambled eggs required a little longer to leave the stomach than boiled eggs. Fried eggs were handled as readily as soft boiled eggs or any other type of cooked egg. Eggs scrambled or fried with a large excess of fat remained in the stomach a little longer, the difference being most marked with the rapid-emptying type of individual. The belief that fried or moderately greasy foods give the stomach appreciably more trouble than others was not supported by our findings.

The response of the stomach to plain and Spanish omelets was found to be quite similar. Omelets remained in the stomach as long as scrambled eggs and longer than boiled eggs. Poached eggs, shirred eggs and soft cooked eggs were found to be among the more readily digested forms of eggs.

Eggs pickled in vinegar were digested in the same time as the hard boiled eggs from which they were prepared. Deviled eggs remain in the stomach a little longer than plain boiled eggs.

The eggs of the duck and turkey are handled by the stomach in the same way as hens' eggs, evacuation being somewhat delayed in the case of turkey eggs due apparently to their greater bulk.

The Chinese preserved egg called "pidan" gave rise to delayed and low acid responses in the stomach as well as delayed evacuation. This may have been due in part to the unappetizing character of these eggs.

Cold storage eggs, whether boiled or fried, could not be distinguished from fresh eggs as far as the response of the stomach was concerned. The same was true of the mixed frozen eggs of commerce, whether these were scrambled or used in the baking of cakes.

Eggs with milk, or egg-nog, leave the stomach a little more slowly than eggs alone, the egg albumin preventing the formation of indigestible curds in the stomach such as are likely to be formed with milk alone.

Eggs with bread or French toast remained in the stomach a little longer than bread and butter alone but not longer than hard boiled eggs.

Bacon and eggs were taken care of by the stomach almost as readily as fried eggs alone while possessing distinctly higher food value.

Frizzled beef with scrambled eggs were digested as quickly as scrambled eggs alone. Eggs and meat appear therefore to form a desirable combination from the standpoint of gastric digestion.

The authors wish to express their appreciation of the coöperation of the students of Jefferson Medical College, who kindly acted as subjects of these tests.

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STUDIES ON THE BRAIN STEM

I. Regulation of Body Temperature in the Pigeon and its Relation to Certain Cerebral Lesions

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INTRODUCTION

For more than a century the pigeon has been a favorite animal for physiological experiments involving the extirpation of various parts of the brain. Although the names of Rolando, Flourens, Vulpian, Munk, Ewald, Ferrier, etc., at once arise to mind in connection with such work, the interpretations of results are in many cases still in an unsatisfactory condition. One factor that in the past has not been properly appreciated is the one which is being emphasized by the new school of comparative neurologists, namely, that the anatomical relationships in the cerebral lobes of the bird brain are not the same as in the mammals. All published data on this subject at present are rather fragmentary and the morphology of the pigeon cerebrum is a thing of doubt and difficulty. Furthermore it is a notorious fact that few careful anatomical studies have been made in connection with physiological studies on the bird brain. (A few experiments have been made in this direction by McKendrick and by Edinger, with the Marchi method.) A better statement of physiological experiments on the pigeon brain still awaits the analytic work of the comparative neurologist on this common laboratory animal. The attempt is being made by the writer to correlate the physiological and anatomical results of brain lesions in pigeons in a more exact way than has been done hitherto.

Anatomical details are not considered in this paper aside from those of the great subdivisions of the brain. In order to illustrate more objectively the location of the lesions referred to, two figures are included of sagittal sections of the pigeon's brain (figs. A and B). There will be published elsewhere a detailed account, in so far as this is possible with present histological technique, of the parts of the brain that seem to be functional after operation.

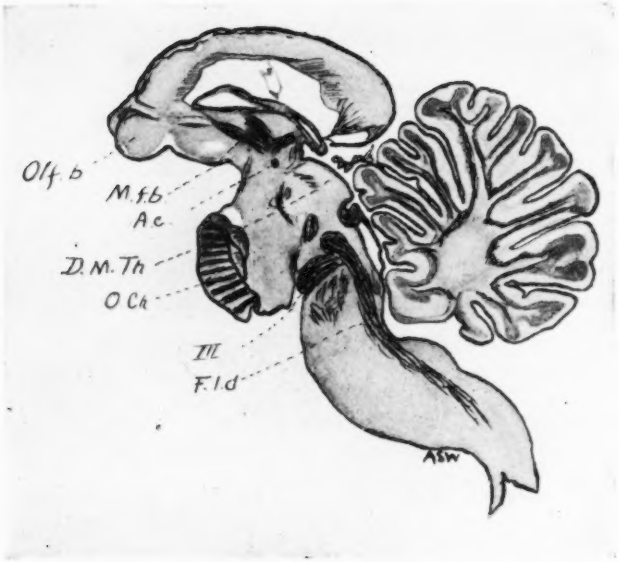

Fig. A. Sagittal section of pigeon brain very near the median plane. Stained with iron hematoxylin.

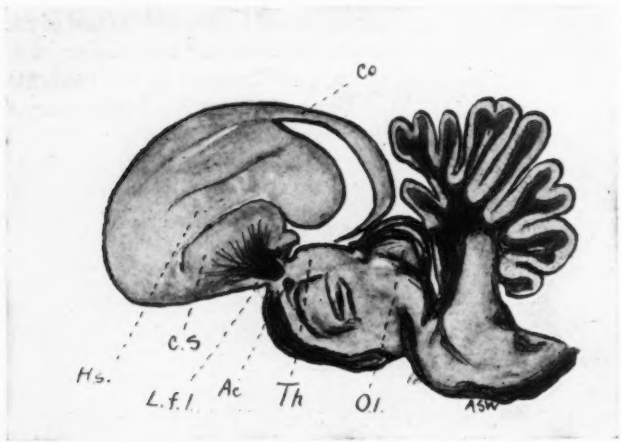

Fig. B. Lateral sagittal section of pigeon brain.

By the term decerebration is meant a section through the forebrain bundles between the striatum and the thalamus. *A.c.*, Anterior commissure; *Co.*, Cerebral cortex; *C.s.*, Corpus striatum (mesostriatum of Edinger); *DmTh.*, Dorsomedian nuclei of thalamus; *F.l.d.*, Medial longitudinal bundle; *H.s.*, Hyperstriatum (Edinger); *L.f.l.*, Lateral forebrain bundle; *M.f.b.*, Medial forebrain bundle; *O.ch.*, Optic chiasma; *O.l.*, Optic lobes; *Olf.b.*, Olfactory bulb; *Th.*, Thalamus.

In a previous paper on the hunger mechanism of the pigeon, the nature of decerebrate restlessness had to be considered. The writer there expressed the opinion that there seem to be variations of reflex excitability, *a*, in different decerebrate birds; *b*, in the same decerebrate bird at different times. This led the writer to make an extensive series of decerebrations to study the *variations* that followed in different animals when the attempt was specifically made to produce the same kind and extent of lesions in all cases. The results may be divided into three groups. The method of decerebration is described by the writer in this Journal (1).

Birds which lived less than two weeks after decerebration. In this group the writer has records of twenty-four birds. Examples of their behavior follow.

Bird 9. Lived 7 days. Nystagmus of head present, digestion seemed to be normal, feathers slightly fluffed. Great difficulty in maintaining equilibrium, stood on its claws with head and body drawn forward, or it fell backwards. Finally spread its wings and rested on its breast, tipped forward. Temperature subnormal, falling as low as 27°C. Autopsy showed cranial cavity filled with a big clot and slight traumatism of extreme anterior and lateral parts of thalamus.

Bird 12. General equilibratory disturbances, which *might* (?) be accounted for by a blood clot on cerebellum found at autopsy. Died second day after decerebration.

Bird 5. Feathers fluffed, no disturbances of equilibrium; listless in appearance, eyes always closed except when touched. No vomiting, no spontaneous movements. Lived ten days.

Bird 16. Slight equilibratory disturbances, feathers flat, occasional violent forced flying movements directed toward the right. Vomiting. Lived 6 days.

Almost every conceivable mixture of variations of this sort may occur —forced movements or immobility; feathers flat against the body or fluffed; nystagmus of head and distortion of neck; body temperature normal or subnormal; restless movements with crop empty and forced movements independent of the digestive conditions; birds become comatose and die.

Decerebration with no visible damage to thalamus. The second group comprises nineteen birds in which there was decerebration with no damage done to the thalamus detectable by naked eye observation. This is the group of so-called typical decerebrate birds; birds which show no forced movements, body temperature normal, restless walking movements when the crop is empty, and a sleepy attitude the remainder of the time. These birds lived for periods varying from three weeks to eight months.

Decerebration with visible damage to thalamus. The third group comprises eleven birds in which there is decerebration combined with visibly distinct damage to the thalamus. These birds are commonly supposed to differ from the classic decerebrate bird only in the absence of spontaneous movements. The birds of this group lived periods of time varying from two weeks to three months. More detailed description of this group will follow later.

Why these variations in physiological effects when the attempt was made to make the lesion identical in all cases? The interpretation given in the past has been that in different cases different reflex pathways have been injured; that in the thalamus is a subcortical center from which nerve pathways arise or terminate that induce certain modifications of body behavior. In how far this is true is a question that, as Tigerstedt says, "cannot for want of critical attention be definitely stated." In part the suggested explanation may be true. There must be some anatomical basis of the differences observed but equally certain is it that one big physiological factor has been wholly omitted in the few considerations of the subject available. As will be pointed out in this paper, decerebration with simultaneous thalamic involvement leads to a condition in which the animal loses the ability to maintain and regulate its body temperature. With these variations in body temperature there is a marked change in reflex excitability of the animal so that, in part if not wholly, the behavior of the animal is dependent on its body temperature. To elicit information dealing with this phase of the subject it therefore became necessary to determine the factors responsible for the variations and regulation of body temperature. This therefore forms the first paper and the application of this to reflex activities will be given in the following one.

TEMPERATURE VARIATIONS OF THE NORMAL BIRD

The body temperature of the normal pigeon varies usually from 40° to 42°C. as measured in the cloaca. Occasionally it may be a trifle less or a trifle more. According to the writer's tests the extreme limits may be considered as from 39.5° to 42.5°C. It is frequently observed that if the thermometer bulb be pushed beyond the cloaca into either the rectum or oviduct that the temperature of 43° to 43.5°C. may be recorded and withdrawal into the cloaca is followed by a fall to 42° or 42.5°. It therefore follows that in any careful comparative measurements the thermometer bulb must always be inserted into the cloaca a

constant distance for a constant time or always be subject to slight uncertainties. In the work here recorded because of this slight uncertainty the figures given at any time are always considered subject to a possible error of 0.5°C.

The variations in the normal bird have been described specifically by several workers. Thus Feré (2) found the normal temperature to vary from 41° to 42°, falling slightly when the animal is quiet, sleeping or incubating eggs, and rising slightly with excess muscular or nervous activity. Furthermore he considered that there is a slight variation according to sex and age, being higher in the male than in the female and higher in the young than in the older bird. Simpson and Galbraith studied the diurnal rhythm and found it similar to that of the mammals. Hilden and Stenbäck (3) have made a comparative study of the body temperature in various birds and confirm the statement of Simpson and Galbraith that, as a general rule, the range of diurnal variations is greater in smaller animals than in larger ones. They also state that by putting the birds in cages under artificial illumination the diurnal rhythm may be reversed and under such conditions the body temperature averages higher than in birds under normal conditions. The diurnal variations described may be readily confirmed and vary from 1° to 2°C.

Variations with external temperature. Exposing a normal bird to extremes of hot and cold, still compatible with life, produces little or no change in the body temperature. Thus sudden changes in the temperature of the bird's cage, varying from 4° to 38°C. for periods as long as twelve hours produced no changes greater than those which may occur in the limits of the diurnal rhythm (fig. 1).

The influence of starvation. Depriving the normal bird of food but not water for a period of five to seven days has little effect upon the body temperature. But during the second week of starvation the body temperature becomes subnormal, and after fourteen or fifteen days may be as low as 36°C. with the bird in a cage at the usual room temperature of 20° (fig. 2, A). During this stage of starvation the bird begins to lose its ability to maintain a constant body temperature and acts somewhat like a cold-blooded animal. Thus, at this time, exposure of the bird to cold is followed by a fall, and exposure to warmth is followed by a rise in body temperature. It is therefore possible by prolonged starvation of the pigeon to reduce it to a condition in which its body temperature is, in part, a function of that of the environment. In all such experiments care has been taken that the starving bird shall always have plenty of water. Feeding the bird promptly brings the

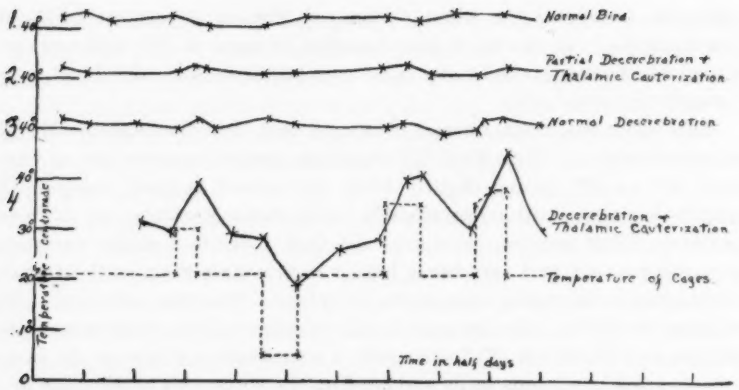

Fig. 1. Time in 12 hour intervals as abscissae; temperature in degrees Centigrade as ordinates. Temperature of cage in broken lines and of birds in continuous lines. Comparison of body temperature variations of four birds with the brain lesions indicated when all were simultaneously exposed to the atmospheric temperature variations of the cage indicated. *1*, Normal bird; *2*, bird with thalamic cauterization after partial decerebration; *3*, normal decerebration, thalamus intact; *4*, complete decerebration and thalamic cauterization.

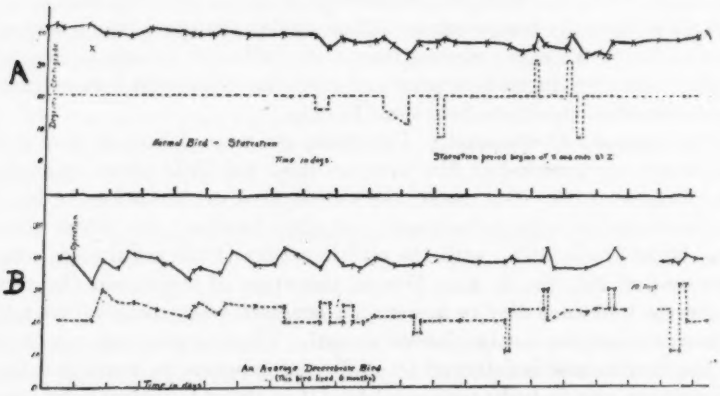

Fig. 2. *A*. Body temperature variations of a normal bird, deprived of food but given water. Time in days; temperature in degrees Centigrade. *B*. Temperature variations of a decerebrated pigeon in which particular pains were taken to make certain that no part of the cerebrum remained in the cranial cavity. Thalamus intact. Temperature of cage in broken lines; birds, in continuous lines.

body temperature back to normal if, of course, starvation is not pushed until death is imminent.

The influence of variations in water intake on the body temperature will be reported later.

TEMPERATURE VARIATIONS AFTER CEREBRAL LESIONS

Temperature studies on decerebrate birds have been carried out by Fredericq (4) and by Corin and Van Beneden (5). The former found that decerebration caused little or no disturbance of body temperature. The latter workers studied the diurnal variations and the carbon dioxide excretion as an index of heat production. They found a possible diurnal variation of 2.2°C., extreme limits of variations in normal birds of 39° to 43.6°, and that after decerebration the carbon dioxide production per kilo body weight is practically identical with that of the normal bird.

The writer's first results were rather confusing. But it finally seemed to appear that the results obtainable could be divided into four groups as follows:

a. Traumatism and pressure on the hemispheres.

b. Decerebration with careful preservation of the thalamus.

c. Decerebration with thalamic lesion.

d. Thalamic lesion with partial destruction of the cerebral hemispheres.

Traumatism and pressure on the hemispheres. In a series of four birds the hemispheres were exposed and roughly pierced in several places by forceps until the base of the cranial cavity was reached. At the same time the longitudinal and transverse sinuses were sectioned. The skin was then sutured over the bloody mass so as to compel the formation of a heavy clot over the hemispheres and cerebellum in addition to the traumatism. In spite of this rough treatment no marked alterations in body temperature occur, nor does the bird lose its ability to maintain a normal body temperature with wide variations in the temperature of the cage (fig. 3). Thus in the case figured although the bird was exposed to a temperature of 5° to 10°C. for a period of twelve hours, there was a fall in body temperature of 2°, a fall within the limits of normal variation.

Effects of decerebration. The results here reported are of those animals only which lived from one to five months after the brain lesion was made. The changes in body temperature after decerebration vary strikingly according to whether or not the thalamic nuclei are simultaneously

damaged. In a careful decerebration, avoiding excess hemorrhage and cutting through the base of the corpus striatum in such a way as to avoid direct traumatism of the diencephalon, it is found that the ability of the bird to maintain a normal body temperature in all variations of external environment is little or not at all affected (fig. 4 B). The bird maintains a normal temperature when exposed to cold and when exposed to heat may exhibit a slight rise, but one still within the limits of normal variation. Two cases have been found after this type of operation where there is an immediate temporary rise in body temperature. This, when it occurs, is striking (fig. 4 B) but no cases have been seen where this rise is distinctly greater than the upper limits of the normal variations. This is in harmony with the recent results of Moore (6) on the rabbit.

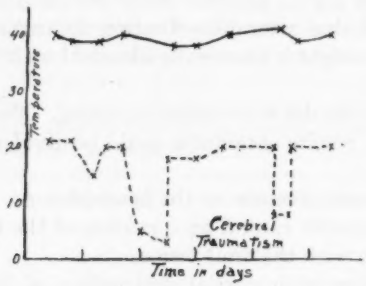

Fig. 3. Temperature variations of a bird after traumatism of the cerebral hemispheres. Temperature of the bird in continuous lines and of the cage in broken lines.

LESIONS OF THE THALAMUS

Decerebration with thalamic lesion. After a clean cut decerebration when the hemorrhage has been controlled, a clear view of the anterior and dorsal surfaces of the thalamus and of the third ventricle may be obtained. In a series of twelve birds a bilateral cauterization or traumatism of the dorsal and medial parts of the thalamus was done with a hot, sharp pointed probe immediately after decerebration. The cautery was applied after a clear view of the structures was obtained, using the habenula and third ventricles as landmarks. After this proceeding the birds commonly die within a few days unless care is taken to keep them warm. In other words, the prospects of such birds living is better in the summer than in the winter if the birds are kept at atmospheric temperature. If kept at a temperature of 25° to 30°C. they may be kept alive

indefinitely, very much as other decerebrate birds. After the operation described, the bird is reduced permanently to a cold-blooded condition (fig. 4A). Its body temperature seems to be largely a function of the temperature of the external atmosphere. Under such conditions its temperature can be arbitrarily set at any level and kept fairly constant by controlling the temperature of the surrounding cage. The temperature of the bird is not that of the cage, but averages 8° to 10° above it. By cooling the cage it is possible to throw the bird into a state of inac-

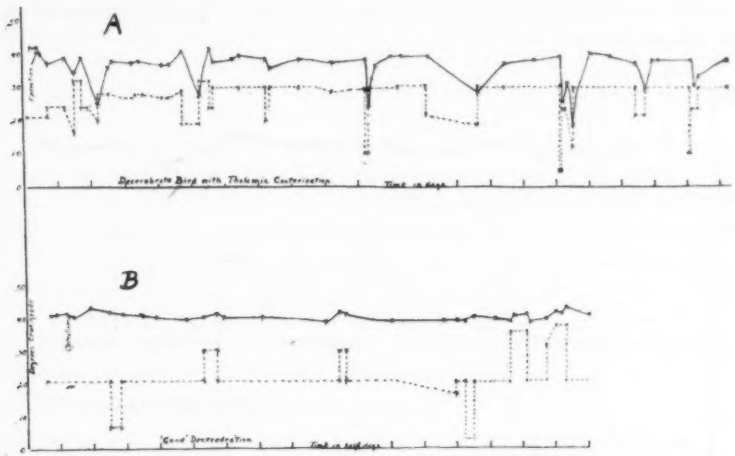

Fig. 4. *A*. Temperature variations of a bird with combined decerebration and thalamic cauterization when exposed to variations of atmospheric temperature indicated by the broken lines. Time in days and temperature in degrees Centigrade. *B*. Temperature variations of a good decerebrate preparation with no detectable trauma of the thalamus. Temperatures of birds in continuous lines and of cages in broken lines.

tivity or immobility that superficially resembles hibernation. By raising the temperature of the incubator the bird is thrown into a condition of hyperpyrexia. The temperature may be thus raised to 45° or 46°C. and death soon follows. The extreme limits to which a single bird's temperature has been pushed without death are 19.5° to 45.5°, a total variation of 16°C. The effects of these wide variations in body temperature on the behavior of the animal will be described in another report.

Thalamic lesion with partial decerebration. The preceding facts suggested of course that there must be some kind of mechanism in the brain stem of the bird that regulates its body temperature. When, however, further attempts at localization were made, surprising results followed. If the two occipital regions of the hemispheres are removed, taking care to leave intact the large superficial cortical artery which runs over the anterior two-thirds of the cerebral lobes, and then the dorso-medial portions of the thalamus are cauterized, it is found that there is no appreciable alteration of the body temperature or in the ability of the bird to maintain a constant body temperature at all temperatures of the environment (fig. 5).

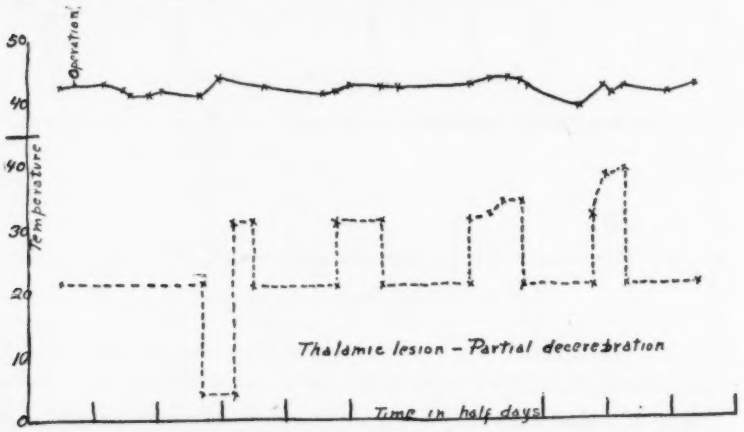

Fig. 5. Temperature variations of a bird with cauterization of dorso-median parts of the thalamus after removal of the posterior parts of the cerebral hemispheres. Temperature of bird in continuous lines and of the cage in broken lines.

The result therefore is that the maintenance and regulation of the body temperature may remain nearly normal—*a*, after extensive traumatism of the cerebral hemispheres complicated with subdural clots; *b*, after decerebration leaving the thalamus intact; *c*, after thalamic lesions leaving the anterior halves of the cerebral lobes in situ. On the other hand, a combination of decerebration and thalamic lesion reduces the bird to a poikilothermous condition. It therefore follows that in what is intended to be a simple decerebration, if there be temporary thalamic involvement, mixed results may follow as seen in the case of figure 2 B. This is characteristic of the average decerebrate preparation

where great care is taken to make certain that all the cerebrum has been removed. There is a period following operation when the temperature is inconstant and varies with that of the environment, but gradually this stage wears off and the animal regains the ability to maintain and regulate its normal temperature.

MINOR VARIATIONS IN THE DECEREBRATE BIRD

Although the decerebrate bird can maintain a normal temperature several differences between it and the normal bird are evident. In the decerebrate bird the range of diurnal variation may be greater than in the normal bird. Thus in a decerebrate bird three months after operation the body temperature during the course of the twenty-four hours varied from 39.5° to 42.2°, a variation of nearly 3°C.

The normal bird put in extreme cold may react by a rise in body temperature of about 1°C. I have never seen this in the decerebrate bird. A decerebrate bird exposed to sharp variations in the temperature of its cage may give corresponding variations in its body temperature which are rather sharp although still within the limits of the normal variation (fig. 1). This the normal bird does not do. (Possibly this may happen during sleep. It has not been tested.) If there be any variation it tends to be compensatory in character, falling slightly on exposure to heat or rising when exposed to cold.

Thus in a normal bird:

TIME	TEMPERATURE OF BIRD	TEMPERATURE OF CAGE
	degrees C.	*degrees C.*
10 a.m.	42.3	22
2 p.m.	42.8	22
6 p.m.	42.0	22
10 a.m.	42.5	22 and raised to 30°C.
2 p.m.	41.0	30 then lowered to 22°C.
6 p.m.	40.5	22
7 a.m.	40.5	22 then lowered to 5°C.
2 p.m.	42.2	5 then raised to 22°C.

The absence of this finer compensatory mechanism, quite variable indeed even in the normal bird, seems to be lacking in the decerebrate preparation. These finer reactions seem to be due to two simple facts. Sudden exposure of a normal bird to extreme cold leads to restless

struggling which may readily account for the slight rise in body temperature. On the other hand, exposure to heat (38° to 40°C.) leads to panting and forced breathing. In the decerebrate bird under the same conditions, cold does not directly stimulate restlessness nor does heat lead to panting. These are important factors the loss of which accounts for some of the irregularities described.

DISCUSSION

A further analysis of these and other possible factors such as alterations of blood distribution and changes in the feathers will be published later. Some of the effects described might be attributed to "shock." Shock, however, is a condition unknown in birds. Certainly, the corpus striatum may be removed without much change in body temperature regulation and equally certain is it that lesions of the thalamus which do not completely separate the hemispheres from the brain stem have little effect on the temperature regulation. It may be that in part the effects are due to altered circulatory conditions in the brain. The temperature alterations could not, however, be produced by pressure of blood clots alone on the hemispheres. There is the possibility that damage to the third ventricle has led to alterations in the pressure relations of the cerebro-spinal fluid and this may produce changes in the medullary centers which play a part in the regulation. Or it may be a combination of changes in both the external and internal cerebral fluid media.

SUMMARY

The normal pigeon exposed suddenly to temperatures of from −4° to 38°C. is able to maintain its body temperature constant within the limits of the diurnal variation. Within these limits, exposure to cold may lead to a slight increase and exposure to heat a slight decrease in the body temperature.

Prolonged starvation of the pigeon leads to a condition in which the body temperature becomes subnormal and rises or falls according to corresponding changes in the temperature of the external air. Feeding the bird when this stage has been reached quickly brings the body temperature back to normal.

Two compensating factors in the normal bird which tend to maintain the body temperature constant in extremes of heat or cold are: excess muscular activity and rapid, forced breathing or panting. Neither of these two factors appears in the pigeon after decerebration.

In the decerebrate pigeon with minimum damage to the thalamus, a normal body temperature is maintained in spite of variations of external temperature of from 5° to 38°C. Under such conditions the body temperature rises or falls accordingly, but only within limits of the normal diurnal variation.

Decerebration and thalamic cauterization reduce the animal permanently to a condition where the body temperature is in large part a function of the external atmospheric temperature. In such animals the body temperature may be lowered to 19°C. or raised to 46°C. by variations of atmospheric temperature of from 10° to 38°C. This renders possible the production in the pigeon of conditions resembling hyperpyrexia on the one hand, or "artificial hibernation" on the other.

Lesions of the dorso-median grey matter of the diencephalon which do not completely separate the fore-brain from the mid-brain, produce little effect on body temperature maintenance or its regulation.

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THE PHYSIOLOGICAL SIGNIFICANCE OF THE REACTION OF TISSUE CELLS TO VITAL BENZIDENE DYES

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Although the results of vitally staining living animals with the acid benzidene dyes are well known, and the distribution of the dye in the body has been carefully studied under normal and pathological conditions, until very recently little attempt has been made to show experimentally how the color enters the cells in which it is found or in what state it exists in the cell cytoplasm. As yet, none of the theories which have grown up about the origin and significance of the stained granules with which the cytoplasm of the "pyrrhol cells" is studded has been generally accepted, and none can be said to have been proven.

Ehrlich (1) saw in the behavior of body cells toward injected dye solutions evidences of the existence of a specific chemoceptor, possessed by certain chromophil elements, which furnishes a bond between part of their cytoplasm and the stain. Goldman (2) concluded that the evidence accumulated in the course of his masterly series of experiments points to the demonstration by the dye of a secretory activity on the part of the pyrrhol cell. Tschaskin (3) believes that the benzidene dyes, isamine blue and trypan blue, color the mitochondria of the various trypanophil elements, and that the great dye masses seen in the clasmatocytes are the result of elaboration of a secretion granule by the chondriosomes of these cells: a secretion which is also stained by the vital azo dye-stuffs.

The most generally accepted theory in this country at present is that advanced by Evans and Schulemann (4) as the result of comparative experimentation with a large number of azo dyes. They believe that vital staining with dyes of this class is the result of phagocytosis by

[1] It is a pleasure to express here my gratitude to Prof. R. G. Harrison for permitting me to use the Osborne Zoölogical Laboratory and its equipment and for advice and assistance which have been of inestimable service.

special cells of ultramicroscopic dye particles, which, though apparently in molecular solution, exist in reality in a state of fine dispersion as an hydrosol. According to these authors any dyes which can be dispersed in particles sufficiently fine to pass readily through the blood-vessel walls are possible vital stains, and they have shown that the presence of multiple sulphonic acid radicles in the dye molecule is favorable to this condition. Dyes of this sort, when introduced into the circulation, pass out through the vessel walls into the tissues and invade them as a foreign body. As such, the dye is removed and segregated by a group of cells, morphologically different, but functionally united—a great scavenger tissue, the units of which Evans calls makrophages. Of course, neither the term "makrophages," nor the word "phagocytosis" (by which Evans means the collection and segregation of the vital dye), are used within the limits assigned these words by custom. The dye particles are probably not engulfed by the process of protoplasmic flowing which is the ordinary conception of phagocytosis, and many of the cells which are "trypanophil" are not phagocytes in the usual sense of the word. On the other hand, some of the cells which are most active in englobing bacteria and other coarse particles have under ordinary conditions no part in the segregation of vital dyes in the body of the living animal, e.g., the polymorphonuclear leucocytes.[2]

Hoffman (5), working in Aschoff's laboratory, has shown that if bits of embryonic liver were cultivated in the blood plasma of an adult animal, drawn immediately after an injection of trypan blue (plasma containing a large amount of dye in suspension) certain cells could be found in the new growth about the transplant which had in their cytoplasm larger quantities of the blue stain than did the majority of the new growing cells. These trypanophil elements were, he said, star-shaped, and the trypan blue existed in their cytoplasm as large masses or coarse granules, contrasting sharply with the fine blue granulations seen in the spindle-shaped "fibroblasts" which formed the mass of his newly grown tissue. Hoffman believed the coarsely granulated cells to be young phagocytic endothelial cells descended from those which line the liver capillaries (Kupfer cells).

[2] In this connection it is interesting to note the description by Downey of vitally stained granules in lymphocytes and granular leukocytes in the blood in doubly ligatured veins and about the site of injections of trypan blue. The author has seen vitally stained lymphocytiform cells in the circulating blood of frogs under pathological conditions.

Now the possibility of staining cells intra-vitam, which can be kept growing under conditions which facilitate the observation of single elements over long periods of time, offers a unique opportunity for studying the reaction of living cytoplasm to the dye, an opportunity which no examination of sections and film preparations from the tissues of vitally stained animals can give. It should be possible to observe an unstained cell in such a culture, to watch the first appearance of color in the cytoplasm and to follow the process of staining until the color has reached its maximum depth and intensity and the individual masses their greatest size and number in the cell body. It should be possible to observe the formation of the vacuoles which can sometimes be seen about the colored bodies and such an examination should throw light on how the dye enters the cell and answer the vexed question as to whether or not the colored masses in the cytoplasm of the pyrrhol cells, after vital staining, are merely aggregates of pure dye or whether the dye-stuff stains a preëxisting cell structure. Should the latter be the case, it ought to be possible to identify this body and to determine its physiological significance.

The technique of making cultures of cells in vitro has been so often described that it is unnecessary to go into it in detail here. These studies were made on the cells of chick embryos cultivated in plasma of healthy adults. The method of making the cultures was that devised by Harrison (6) and modified by Burrows (7), used almost exactly as the latter originally described it. The heart and liver were removed from chick embryos to Locke's solution with a needle and Noyes iridectomy scissors, and cut into bits under the binocular microscope. The fragments, with a small amount of the saline solution, were then transferred to a sterile cover slip and covered with a thin layer of plasma. The cover glass was sealed over the chamber of a deep hollow slide with a ring of vaseline and the culture so made was incubated at a temperature of 39°C.

The instruments, glassware, etc., used were previously sterilized, and sterile technique was observed throughout the operation. The plasma was handled in paraffined glassware and kept in an ice-salt freezing mixture. Great care must be used to prevent evaporation of fluids, since the consequent concentration interferes seriously with successful growth of the explanted tissue.

The first cultures studied were made of somatic cells of the chick embryo in the plasma of blood drawn from an adult hen one minute after intravenous administration of a 5 cc. dose of a 1 per cent solution

of trypan blue. When this blood was centrifuged, a beautiful deep blue plasma solution of the dye could be pipetted off from the corpuscles at the bottom of the centrifuge tube. It was soon found, however, to be more convenient to introduce the dye into the culture medium after the latent period and the initial rapid growth of the explanted tissue had passed and the new growth had become more stabile in its new surroundings. In succeeding cultures a solution of the dye in isotonic salt solution was added to the plasma clot in which the culture was growing after growth was well under way. This operation was done when the cultures were twenty-four to forty-eight hours old, and was carried out by injecting the dye solution into the plasma medium from a very slender curved pipette passed through the vaseline ring which sealed the cover glass to the hollow slide in the chamber of which the cultures were growing.

By the above described method, studies were made of the reaction of cells to the benzidene dyes, trypan blue, trypan red, Congo red, azo blue and benzopurpurin, to colloidal silver and manganese, to carbon granules of various sizes and other particulate foreign material. Cell cultures were supravitally stained with brilliant cresyl blue, neutral red and janus green and studies were made of cells stained with mixtures of two or more azo dyes and various combinations of benzidene vital staining and granule phagocytosis with supravital coloration. All the findings were controlled by observations on the living tissues of adult animals prepared for examination by appropriate methods.

In the young cells which grow in a thin membrane or in individuals which have separated from the tissue mass in such a culture and wandered off on the cover slip, the protoplasm is spread abroad in a very thin sheet, and cells in this condition were selected for examination, since in those growing deep in the clot along fibrin strands the cytoplasmic structures are crowded so closely together that it is difficult to make out detail in the mass.

The cytoplasm of these young cells contains beside the homogeneous, clear nucleus with its one or more grayish nucleoli, fat droplets, mitochondria and, in parenchyma cells from the liver, pigment and secretion granules. The central body, centriole, centrosomes, etc., cannot be seen as clearly as in the cells grown in salt solutions described by Lewis and Lewis (8) though their situation can usually be made out. Mitochondria are small, grayish, refractile bodies of varying shape and size and in plasma grown cells they are usually filaments, straight or curved rods or small granules. They have the power of changing from one to

another but they are only very slightly if at all motile in cells grown in plasma in contrast to the rapid, vigorous movements they make in the cytoplasm of cells planted in salt solution. They are selectively stained a beautiful blue green color by 1: 10,000 solution of janus green, a dye which quickly indicates the mitochondrial nature of a doubtful granule.

Besides these bodies, cells in twenty-four hour plasma culture have another granulation which is sometimes impossible to distinguish in unstained cells from the granular forms of mitochondria. In these young cells there are very small granules which may or may not be seen to be surrounded by a small vacuole in the cytoplasm. They are very active at this stage and they move about in the cell freely, and very rapidly. They may dart along or across the cell, and may, after an interval of rest, return to their original position or they may go off in another direction. Frequently they travel along the length of the cell by short tacks from side to side. Movement is irregular and always jerky in character. Sometimes in the course of this movement, the vacuole enclosing the granule may be drawn out into a long thread which, when the granule comes to rest, reassumes its normal relation to it, or the thread may break and the vacuole round up without its granule, or the vacuole may disappear into the surrounding cytoplasm. I have never seen two vacuoles unite to form one.

Now if one examines an older culture, say a forty-eight hour or sixty-hour one, a change in some of the cells is at once evident. Cells may be found whose cytoplasm is packed with large grayish bodies on which pressure has forced the most varying and bizarre forms. They may be round or oval or angular or indented in every imaginable way. Sometimes the cells are full of them, sometimes a cell may contain only one or two. Sometimes in a single cell one finds all transition forms between the tiny motile granules seen in all young cells and the huge masses just described, and it is evident that the two forms are identical and that the large granules are the result of the growth of the small ones, though the large granules are non-motile and usually show no sign of a surrounding vacuole. These large granules are not contained in all cells. Many of the mesenchymal elements up to the death of the culture never contain any but the small type of granules. On the other hand, transition forms between the two types may occur.

The identity of these granules is further assured by the evidence of cultures stained supravitally with neutral red or cresyl blue. The motile granules and the large masses stain a brilliant red with the first named dye and a deep purple with the second. The vacuoles surround-

ing the small granules are colored rose-pink by cresyl blue and brick red by solutions of neutral red. Mitochondria of the active living cell are never stained by the above mentioned dyes but the mitochondria of a moribund or dead cell may be brilliantly colored by either. Even in supravitally stained preparations it is often not possible to see the vacuole about the large bodies; but some of these masses are found, at one side of which a narrow, stained, crescentic segment of the vacuole which surrounds the granule is visible. In other words, the vacuole is usually entirely filled by the swollen granule.

The reaction of these structures to supravital stains is sufficient to identify them with the cytoplasmic vacuoles and neutral red granules which Lewis and Lewis (8) have described in tissue culture cells and which they once interpreted as a degenerative change in the cytoplasm. In cells grown in plasma, however, the granule is the prominent feature of the picture given by supravital staining, instead of being a tiny dot swimming about in a huge vacuole as it is in cells grown in salt solutions. This difference in size relation between the vacuole and the segregation granule which it encloses results from the difference in the media in which the cells are growing. Living protoplasm in a glyco-saline medium is on a diet which, while sufficient to support life for a time, is extremely poor in particulate matter. In other words, the cells in saline media are living in molecular solutions. Plasma grown cells, however, are growing in a medium rich in colloids of various sorts. The significance of this environmental difference will appear later when the relation of foreign particles to the growth of the "neutral red" granule is made plain.

The so-called "neutral red granules" which the Lewises (8) have described have without a doubt the same physiological significance as the structure which they call the vacuole. Their neutral red granules are identical with the small type of neutral red granules which are found in plasma grown cells.[3]

If film preparations from the subcutaneous tissue of adult animals are examined, these same bodies may be found in all the connective tissue cells. They have the same appearance in unstained films and the reaction of the granule to neutral red and to cresyl blue is identical with that of the granules in culture grown cells. In adult tissues the large granules are found almost entirely in the cytoplasm of the clasmatocyte;

[3] In a later paper published in collaboration, Mrs. Lewis has established the identical nature of the vacuoles and neutral red granules which she and W. H. Lewis described previously in cells grown in vitro in saline solutions.

the fibroblast, on the other hand, being characterized by the possession of the small variety; but in the connective tissue of the adult animal, as in cultures of embryonic cells, it may be extremely difficult to draw a hard and fast line between the two cell groups, and transition forms between the two extremes may sometimes be found. It is very evident that these granulations are the granules which Maximow (9) and others have described in connective tissue cells after supravital staining with neutral red, and there is no doubt that the stainable masses in these culture grown cells represent the vacuoles and granules on which Renault (10) has based his theory of secretion by the "mode rhagiochrine"—the vacuoles and granules of segregation.

That is to say, we have to do here with a differentiation in vitro of indifferent mesenchyme cells which form resting wandering cells or clasmatocytes, and the differentiation is a process of elaboration of granules in the cytoplasm.

When the cells are stained by the vital azo dyes, trypan blue, trypan red, etc., the primary diffuse stain in cells is soon localized and, after twenty-four or forty-eight hours, the segregation granules contain all the color. The entire segregation granule may not be uniformly colored at once. Often the stain is first seen at one side of the granule where it forms a narrow crescent of color which broadens until it makes nearly the whole of the granular mass; or the dye may come into the granule equally from its entire periphery, in which case the edge of the granule is seen in optical section as a colored ring. The different stages of these processes of staining of the segregation granule by the dye, account for the crescent and ring shaped granules of dye which have been described in the clasmatocytes of the subcutaneous tissue of vitally stained animals.

All of the large granules in a single cell may not be colored at the same time. Sometimes a cell whose cytoplasm is merely a mass of granules of this sort will have only one or two which have been stained. The number of stained granules in a given cell, however, increases with the length of time the cell is exposed to the dye. The small type of granule takes the stain just as rapidly as the large. Colloidal metals like manganese and silver (collargol), when taken up by living cells, are segregrated and stored within segregation granules which they color.

The possession of segregation granules is not confined to connective tissue elements. They are present in large numbers in the cytoplasm of various parenchyma cells, e.g., of the liver; and exhibit in this situation the same staining reactions, thereby accounting for the blue granulations in the parenchyma cells of the livers of animals stained with

trypan blue. Most of these cells, however, are equipped only with small granulations of the fibroblast type.

The nature of the segregation granule and the reaction of the living cell to the benzidene dyes are best understood after a study of culture grown cells which have been fed with India ink. If carefully filtered India ink is mixed with the plasma used as a culture medium, the clot has a light yellowish brown color and appears homogeneous when examined with the dry objectives. Even with the aid of the oil immersion system, the largest granules of ink are scarcely visible as tiny brownish bodies, and the smallest are so minute as to be beyond the range of microscopic vision. In twelve hours, however, cells growing in such a clot have gathered large amounts of ink and stored it. Black masses, evidently aggregates of multitudes of small granules of carbon, can be seen in the mass of segregation granules and in vacuoles which, though they contain apparently only ink aggregates, give the same staining reactions as those about the ordinary neutral red granule.

The aggregation of submicroscopic carbon particles to form part of the segregation granule together with a study of the growth of the granular mass in the untreated cell leaves no other alternative for the interpretation of the significance of the segregation granule than as a mass of aggregated foreign material which the cell has gathered and stored and to which it is constantly adding albuminous and other submicrons derived from the surrounding tissue fluids where they exist in the colloidal (emulsoid or suspensoid) state.

Vital staining of cells with azo dyes is accomplished by the same process apparently by which the cell acquires and stores other substances in the colloid condition which are the material useful as food to the body as well as those which are mostly debris or actively injurious.

The granule which stains intra vitam in the cell body is then the equivalent of the protozoan food vacuole and since the amicrons of the semicolloidal dyes are stored in it, we have in benzidene vital staining an index and a demonstration of a feeding and storage reaction on the part of the cells of warm-blooded vertebrates.

The analogy between the "food vacuole" of the protozoa and the segregation vacuole and granule in the vertebrate cell is made very evident by staining amoebae and paramecium with solutions of trypan blue. After a varying interval the protozoan segregates the dye which has entered its cytoplasm, in preformed vacuoles which contain the chylomonas or other food which the protozoan has surrounded. The chylomonas may be seen unstained in or beside the aggregated mass of trypan

blue which grows larger as the animalcule remains in the dye solution. Moreover it is a matter of common knowledge that the vacuoles of the protozoan and the contained food mass are selectively colored intravitam by neutral red, and Loele (11) has called attention to the fact that both acid and basic dyes color the "oxydasekugeln" or nourishment vacuoles of paramecium. I may say here that the food vacuoles of amoebidae give the same reaction with brilliant cresyl blue 2B which characterizes the segregation granule and vacuole of the cells of warm-blooded vertebrates, and that a detailed study of these dye reactions in protozoa is now well under way.

Large granules of carbon and coarse granules of the high molecular dye-stuffs which gain entrance to the cytoplasm may become part of a preëxisting segregation granule in the substance of which they may be found embedded, or if they are of sufficiently large size, one or several may be found enclosed in an "inclusion" vacuole which has the same functional significance as a locus for the storage of dye-stuffs and other foreign material as the spaces about those granules of segregation which are visible in the untreated cell. The living cell deals in exactly the same way with bacteria, algae, fat droplets, red blood corpuscles, free pigment, etc., which it has engulfed in its cytoplasm, and it is possible to find in a cell, vitally stained with trypan blue, masses of the azo dye in the same "inclusion vacuole" which contains coarse particles phagocytized by the cell, whether those particles are some extraneous foreign granules like cinnabar or a cellular entity like a red blood corpuscle. Just so we may find pigment and the remains of red blood cells in the masses of trypan blue.

It is well known that basic dyes like neutral red will stain the so-called "inclusion vacuoles" about phagocytized material and will after a time color the inclusion itself, just as food masses and the fluid in the food vaculoes of protozoa are tinted by the same dye. How far fetched are the attempts which have been made to establish a functional differentiation of the neutral red staining granule of the cell's cytoplasm from the food masses and "inclusion" vacuoles on the basis of slight or imaginary differences in color tone after staining with neutral red will be at once evident when we remember that the shade of the neutral red stain may vary in the food vacuoles of the different protozoa and those of the higher metazoan forms whose gastro-intestinal epithelium still retains a recognized intracellular digestive function. In these lower forms the zoölogists recognize the tone differences after supravital staining as due to variations in the chemical composition of the digestive fluid in the

vacuole and the food mass (e.g., variations in amount of acid) but no one would venture to deny for these structures a fundamentally similar functional significance. Careful study of living cells in cultures and in the body of the adult animal and their behavior toward dyes and other organized particulate material shows conclusively that the "inclusion mass" and "segregation granule" are physiologically identical.

In short, all sorts of material which the cell allows to enter, or takes into, its cytoplasm, eventually finds its way into some of these vacuoles of segregation and becomes an integral part of the contained granule. Certainly this is true of particulate substances, and since the subcutaneous injection of distilled water is followed by the appearance of large fluid filled vacuoles in the connective tissue cells apparently identical with those which appear in cells grown in saline media, it seems likely that ingested fluids and solutions may meet with the same treatment in the cytoplasm. Again, some of the protozoa, for example paramecium, are able to concentrate or aggregate the relatively small molecules of phenolsulphonephthalein in their food vacuoles and to retain it for a considerable time under certain conditions.

The aggregation of ultramicroscopic granules of carbon into the neutral red body effectively disposes of the necessity of considering the possession of a dyeceptor by these bodies, to account for their coloration by pseudo-solutions of azo dyes. Even without the evidence for mechanical mixture which is offered by the incorporation of the inert carbon particle in the substance of the segregation granule, it is difficult to think of the gradual progressive process of granule staining by these dyes as the result of chemical combination of the dye with granular substance. Nor is it possible to credit these granules with the possession of a multitude of chemoreceptors capable of binding a range of substances from carbon to trypan blue whose molecules present the most diverse structure and among which there is not the least shade of chemical relation. The exact mechanism by which the cell takes the tiny particles in colloidal suspensions and pseudo-solutions into its cytoplasm is still uncertain; but it is not at all difficult to suppose that we are merely dealing with another manifestation of well-known physicochemical phenomena. The cytoplasm of the animal cell is almost universally recognized today as a colloid system and it is well known that such systems allow the entrance into and solution or dispersion in their dispersion means of substances whose particles have become adsorbed on the system's surface. Such an adsorption of amicrons of dye onto the surface of a cell bathed in a dye solution is only what is to

be expected in the light of the Gibb's law that substances in solution collect at interfaces since surface concentration tends to diminish free energy at such interfaces and under these conditions the adsorption of the disperse phase of a colloid sol is bound to follow.[4]

Under certain conditions this process may be actually seen to occur in tissue cultures. Collargol hydrosols are apparently toxic to the cells of chick embryos in culture and the silver gains access to the cytoplasm only with extreme difficulty. After a short time the bodies and processes of cells which have been treated with the silver colloid may be seen entirely covered and completely outlined by a thick coagulum of adsorbed silver granules.

Included in the cell's cytoplasm, the segregation of dye particles into the vacuole of segregation probably follows along the same lines, for we must remember that the protozoan "food vacuole" and the vacuole of segregation in the vertebrate cell are really no more and no less than fluid droplets, and that where their surfaces are in contact with the cytoplasm we have again an interface between two fluid systems. In vitally stained paramecium, one can sometimes see colored granules concentrated about the food vacuole before the color enters the fluid globule, in which the animal's food undergoes digestion. In this connection it is interesting to note the relation which seems to prevail between the motility of these food masses and that of the cytoplasm. In amoebae, whose cytoplasm is constantly and rapidly flowing in all directions, the food vacuole is passively moved about as the cytoplasm flows past. In the comparatively sluggish current of the protoplasm of the paramecium the food vacuole moves slowly over a route which exposes it at some time during its journey to the entire cell body. In the quiet cytoplasm of the cells of higher vertebrates, however, until movement is automatically mechanically checked by distention of the cytoplasm with masses of stored foreign material, the segregation granule is constantly rapidly and independently motile, and each one on its excursions covers a large part of endoplasm of the cell. It seems hardly necessary to point out the importance of the motility of the granule as an aid

[4] Evans has brought again to our attention a point in the morphology of the pyrrhol cells which was emphasized by Metchnikoff very early in his studies. If these cells are examined during life their periphery may be seen to be covered with tiny spine-like processes of the hyaline ectoplasm which are constantly and rapidly protruded and retracted—tiny pseudopodia. The constant changes in the surface of a cell whose ectoplasm is exhibiting this sort of movement and the resultant increase in the surface available for adsorbing particles must tremendously increase the absorbing capacity of the cell.

to the aggregation into its substance of particulate matter which has passed through the cellular ectoplasm, since by this means the granule wanders over a large portion of the cytoplasm collecting foreign substances as it goes, and, as it were, combs the cytoplasm for the food which protoplasmic currents bring to the vacuole in the amoeba.

Once in the vacuole the growth of the segregation granule becomes merely a matter of coagulation of amicrons to microns and the adsorption of dye granules on each other or on albuminous or other phases which are dispersed in the vacuolar fluid in the same way in which two or more small granules in a single vacuole may be seen to unite to form one. There are no doubt two factors which aid greatly, if indeed they are not entirely responsible for the aggregation of the component particles in the segregation granule—concentration of the colloid in the vacuole by constant accumulation of freshly absorbed particles and by reimbibition of water into the cytoplasm, concentration which alone is sufficient to cause aggregation of the disperse phase in a sol, and as Schulemann (12) has shown experimentally, the presence of electrolytes in the fluid in which the amicrons are dispersed. All of these colloidal dyes are more or less precipitated in the presence of electrolytes in solution.

While the above description is an attempt to picture the course of events which result in the vital staining of a cell by an acid azo dyestuff it holds also for the process by means of which the cell stores up colloidal proteins and other finely divided materials useful in the metabolic processes of the body. Moreover it is questionable whether staining with the "basic dyes" is always the entirely different process which Evans calls it and which at first glance one might believe it to be. Let us take, for example, the staining of the living cell by neutral red. This staining follows the same course in the cell as coloration by one of the benzidene dyes or metallic colloids. A diffuse, gradually increasing cytoplasmic stain is followed by the collection of the dye in the segregation granule and it is only the death of the cell which releases it to stain the nucleus, nucleoli and mitochondria. The same is true of cresyl blue. We have seen previously that even molecular solutions may be concentrated in the fluid surrounding the segregation granule and neutral red is probably not absolutely a molecular solution if its slow diffusion rate (13) may be taken as an indication, and furthermore neutral red may be precipitated from solution by salts. This dye probably hovers somewhere in that indistinct region between the colloid state and a state of true solution, and its sols probably represent

very tiny particles rather than dye molecules suspended in the dispersion means. Of course many dyes like janus green[5] beside entering the segregation vacuole color specific granules in the cytoplasm, and these phenomena, equally of course, are probably of an entirely different nature. But whether they result from a true chemical affinity or from some other cause, we have not yet been able to decide. Again the story of the colloid's existence in the cell is not finished with its aggregation with the food mass, but it often undegroes chemical changes from interaction with substances in the fluid about it, e.g., the metachromasia which follows aggregation of Congo rubin (Schulemann).

At all events the individual granules are certainly segregated in preformed vacuoles, where, by aggregation to like granules and other material, they form the neutral red staining body of the authors. In this way particles of the vital azo dyes are mixed with the segregation granule and color it. They can be said, therefore, to stain a previously existing structure, though the staining is not by a chemical union with a part of the granular molecule but a mixture with the granular substance.

It is very difficult to say with certainty whether or not new vacuoles are ever created in a cell to care for ingested dye-stuffs. That such a process occurs in caring for finely divided particulate matter is doubtful. It is possible that the segregation granule in some of the vacuoles may be

[5] I am not prepared to say what the factors are which govern the rate of absorption and segregation of dye-stuffs by the living cell or the quantitative and qualitative relation of the storage of ingested foreign material. Certainly the rapidity of absorption is not determined by the osmotic pressure of the solutions in contact with the cytoplasmic interface. Trypan red, a dye which is rapidly and readily diffusible is not stored by the cell in appreciable quantities until several hours after its exhibition, while brilliant cresyl blue, which hardly passes at all through semipermeable membranes in four and twenty hours, stains the segregation granule of exposed cells in a few minutes. Moreover, when cells are exposed to trypan blue sols metachromatic granular staining is rarely seen, although this dye is not a distinct entity. Two colors may be separated by diffusion experiments from its sols, one blue, which diffuses slowly, the other, a red, and rapidly diffusible dye. These two colors may represent chemically distinct substances; on the other hand, and this is much more probable, trypan blue may belong to that class of substances described by Michaelis which forms hydrosols in which the degree of dispersion is multiple (polydispersoid). Staining with weak solutions of neutral red is a comparatively slow process but intense coloration of the segregation granule follows almost instantly on the exhibition of a concentrated dye solution, but concentration of solutions of dyes of the benzidene series has no appreciable effect on the rapidity with which vital staining takes place. Consideration of the reason of these facts must suit for a more complete understanding of physico-chemical phenomena, especially those which have to do with adsorption.

made up almost entirely of the dye substance after prolonged feeding with pseudo-solutions of the benzidene colors but it is more probable that the dye is present only mixed with other ingested material. Nor is it well to ignore the possibility that some of the dye may be taken into the cell as an adsorption compound with albuminous or other particles which are always entering the cell from the body fluids. So many of these vacuoles exist in the cytoplasm of a normal cell that it is not necessary to assume that new ones are created to care for injected dye, and besides the large vacuoles filled with swollen granules of segregation, there is always a reserve of tiny vacuoles which are capable of swelling to care for a condition of overfeeding. We know, however, that when large foreign bodies are ingested, they are enclosed in a fresh vacuole formed during their engulfing. It has been already pointed out that such a vacuole is identical physiologically with the other segregation vacuoles normally found in the cells, and it has been shown above that the relationship is at once made evident by subsequent feeding with vital dyes the aggregates of whose amicrons may be seen segregated in the "inclusion vacuole" along with the englobed body whose ingestion brought it into being.

Combination of intra-vitam staining with an azo dye, for example trypan red, with supravital coloration of the mitochondria by means of a solution of janus green, demonstrates in a most striking manner, the distinct individuality of the two sorts of granules; and the same result may be obtained by staining growing cells simultaneously with janus green and neutral red. The mitochondria are not colored by the red dye, which stains the segregation granule, but are specifically stained with janus green. No relation has been observed between mitochondria and segregation granules and they are apparently distinct throughout the existence of the cell in spite of the assertions of Tschaskin (3), Levy (14) and Maximow (15) to the contrary.[6]

[6] I have seen, however, in active, vigorously growing cells, when first exposed to isamine and trypan blue, distinct staining of the mitochondria, which is not permanent. The dye soon leaves these tiny bodies to enter the segregation vacuole and the mitochondria are, in a short time, left colorless. This phenomenon must not be confused with the preagonal staining of mitochondria of moribund cells by solutions of benzidine dyes by which they are surrounded. In such cells the first evidence of impaired vitality is coloration of the mitochondria. This is followed by staining of the nucleoli and the precipitation and staining of a "chromatin network" in the nucleus. The tone gradually deepens and becomes homogenous throughout the cell body, which rounds up, shrinks and finally degenerates into a mass of granular debris. What the meaning of the primary mitochondrial

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It is very difficult to say with certainty whether or not new vacuoles are ever created in a cell to care for ingested dye-stuffs. That such a process occurs in caring for finely divided particulate matter is doubtful. It is possible that the segregation granule in some of the vacuoles may be

[5] I am not prepared to say what the factors are which govern the rate of absorption and segregation of dye-stuffs by the living cell or the quantitative and qualitative relation of the storage of ingested foreign material. Certainly the rapidity of absorption is not determined by the osmotic pressure of the solutions in contact with the cytoplasmic interface. Trypan red, a dye which is rapidly and readily diffusible is not stored by the cell in appreciable quantities until several hours after its exhibition, while brilliant cresyl blue, which hardly passes at all through semipermeable membranes in four and twenty hours, stains the segregation granule of exposed cells in a few minutes. Moreover, when cells are exposed to trypan blue sols metachromatic granular staining is rarely seen, although this dye is not a distinct entity. Two colors may be separated by diffusion experiments from its sols, one blue, which diffuses slowly, the other, a red, and rapidly diffusible dye. These two colors may represent chemically distinct substances; on the other hand, and this is much more probable, trypan blue may belong to that class of substances described by Michaelis which forms hydrosols in which the degree of dispersion is multiple (polydispersoid). Staining with weak solutions of neutral red is a comparatively slow process but intense coloration of the segregation granule follows almost instantly on the exhibition of a concentrated dye solution, but concentration of solutions of dyes of the benzidene series has no appreciable effect on the rapidity with which vital staining takes place. Consideration of the reason of these facts must suit for a more complete understanding of physico-chemical phenomena, especially those which have to do with adsorption.

made up almost entirely of the dye substance after prolonged feeding with pseudo-solutions of the benzidene colors but it is more probable that the dye is present only mixed with other ingested material. Nor is it well to ignore the possibility that some of the dye may be taken into the cell as an adsorption compound with albuminous or other particles which are always entering the cell from the body fluids. So many of these vacuoles exist in the cytoplasm of a normal cell that it is not necessary to assume that new ones are created to care for injected dye, and besides the large vacuoles filled with swollen granules of segregation, there is always a reserve of tiny vacuoles which are capable of swelling to care for a condition of overfeeding. We know, however, that when large foreign bodies are ingested, they are enclosed in a fresh vacuole formed during their engulfing. It has been already pointed out that such a vacuole is identical physiologically with the other segregation vacuoles normally found in the cells, and it has been shown above that the relationship is at once made evident by subsequent feeding with vital dyes the aggregates of whose amicrons may be seen segregated in the "inclusion vacuole" along with the englobed body whose ingestion brought it into being.

Combination of intra-vitam staining with an azo dye, for example trypan red, with supravital coloration of the mitochondria by means of a solution of janus green, demonstrates in a most striking manner, the distinct individuality of the two sorts of granules; and the same result may be obtained by staining growing cells simultaneously with janus green and neutral red. The mitochondria are not colored by the red dye, which stains the segregation granule, but are specifically stained with janus green. No relation has been observed between mitochondria and segregation granules and they are apparently distinct throughout the existence of the cell in spite of the assertions of Tschaskin (3), Levy (14) and Maximow (15) to the contrary.[6]

[6] I have seen, however, in active, vigorously growing cells, when first exposed to isamine and trypan blue, distinct staining of the mitochondria, which is not permanent. The dye soon leaves these tiny bodies to enter the segregation vacuole and the mitochondria are, in a short time, left colorless. This phenomenon must not be confused with the preagonal staining of mitochondria of moribund cells by solutions of benzidine dyes by which they are surrounded. In such cells the first evidence of impaired vitality is coloration of the mitochondria. This is followed by staining of the nucleoli and the precipitation and staining of a "chromatin network" in the nucleus. The tone gradually deepens and becomes homogenous throughout the cell body, which rounds up, shrinks and finally degenerates into a mass of granular debris. What the meaning of the primary mitochondrial

If a culture of cells grown in trypan blue is supravitally stained with neutral red, the blue granules take the red color also and become purple, then violet, then red, as the red dye becomes optically stronger than the blue. Staining of cells with mixtures of trypan blue and trypan red results in the presence in the cells of red granules and blue granules and granules stained violet or purple, as a result of a combination of different amounts of the two stains. It is not necessary to assume the possession of different chemoceptors for different dyes to explain this phenomenon. Since coloration of the segregation granule is due to the presence of dye granules in a mechanical mixture, the tint which the granule assumes after mixed color feeding depends only on the number of dye granules of each kind which happen to be poured into the same vacuole.

Since all sorts of materials which the cell ingests find their way into vacuoles of segregation, it is of course in these vacuoles that the intracellular digestion processes with which we are familiar must be carried on—the lysis of red blood corpuscles, bacteria, etc. The vacuole which originally probably contained only the segregated fluid which the cell had absorbed, apparently serves as a sort of cell stomach, an organulum in which the preparation of various materials capable of serving the cells in their amabolic processes takes place. We may think of the vacuole as a fluid globule into which the various secretions of the cytoplasm are poured, to act upon the mass of the segregation granule within, or upon specific substances which the cell may have swallowed and passed into the fluid globule which is the vacuole of segregation.[7] In these tiny stomachs, materials useful to the cell may be selected and separated from poisonous or useless matter, to be reabsorbed as food by the cytoplasm or passed on to other cells for nourishment or to build

staining may be I cannot say but an analogous process apparently goes on in some animal and plant cells under normal conditions since these granules may be found brilliantly colored by various natural pigments, such as anthrocyanin and chlorophil which has led some authors to use the term "chromochondria" to describe them. (Asvadourova N. Arch. d'Anat. Micr., T. 15, pp. 153-314). It is doubtless this sort of staining which has led Levy and others to believe that the segregation granule is the result of a mitochondrial metamorphosis.

[7] There can be little doubt, moreover, that just as many drugs and other substances (e.g., morphine) are, after absorption through the gastric or intestinal mucosa, reëxcreted into the gastro-intestinal tract, so waste products of cytoplasmic metabolism may find their way into these vacuoles of segregation as well as into the surrounding fluid of the tissue spaces, for it is hardly conceivable that the cell should instantly differentiate between one substance and another of like physical properties in solution.

secretion. In this connection it is interesting to note that reabsorption of material into the cytoplasm of these pyrrhol cells can be shown to occur experimentally. If the phagocytic cells are fed with emulsions of fat or oil stained with Sudan III, the droplets of fat which are taken into their cytoplasm gradually grow paler and at length may lose their color entirely. If this process is allowed to continue uninterrupted, finally all the stain may be removed from the foreign oil and is found coloring the normal fat in the body of the injected animal.[8] This course of events is followed even though the injected oil is an indestructible compound like the petroleum oils of the naphthene series. How far the destruction of fat-laden phagocytes may aid in producing the final effect cannot be told but the growing pallor of the droplets enclosed in the cytoplasm of healthy cells is a certain indication of reabsorption of the dye from the engulfed oil into the cytoplasm of the cell itself, just as is the staining of the animal's own fat sure proof that the dye is given off from the phagocytes to the animal's body. The waste is after a time cast out again as débris into the surrounding tissue fluid or freed in toto together with food products by the destruction of the short-lived and fragile cells which contain them.

Therefore, inasmuch as the vital benzidene dyes are deposited in these organs, the colored masses in the clasmatocytes, etc., after injections of these colored pseudo-solutions, in a way, are also an index of secretory activity on the part of the stained cell as Goldmann and students of the French school have suggested.

The power to carry on these processes is in some degree common to all cells since even the nerve cell contains vitally stainable granules, but certain elements—the Kupfer cell, the clasmatocyte, etc.—are especially active in this direction, and it is probable that we must consider these as a primitive cell type nearly related to unicellular organism. The degree to which the body cells which are not under ordinary conditions included in the class to which the clasmatocytes belong are called upon to perform these phagocytic functions is variable and is controlled by factors of which as yet we have a very imperfect comprehension. Cells of the same type in different animals will manifest very different reactions toward materials in the colloid state introduced into the animal's circulation. For example, Wislocki has found that the liver cells of dog-fish will store amounts of trypan blue as large as those found in the cytoplasm of the Kupfer cells, while the liver cells in Cyprinidae after the same treatment contain no traces of the dye whatsoever.

[8] Shipley and Cunningham, and Cunningham and Wislocki unpublished.

Again under conditions which vary from the normal any cell may be called upon to exercise a latent potentiality for ingestion, storage and digestion, as the lining cells of the blood vessel which normally refuse trypan blue or take it only in small amounts, will, following embolism, revert, functionally speaking, to a primitive type and engulf large quantities of dye-stuff (16).

We are most familiar with the pathological physiology of the "pyrrhol cells"—their ability to destroy bacteria and other invaders which they have ingested, their relation to malignant neoplasm and parasitic cysts. Their importance as collectors of extraneous matter and the part they play in the organism's defensive reaction against foreign bodies is well recognized but we are still much in the dark as to their rôle under normal conditions in the everyday life of the body. In reactions to pathological stimuli, we are dealing only with an exaggerated manifestation of a normal physiological digestive reaction which has been only slightly modified to meet an emergency (formation of giant cells, etc.). To dismiss these cells as scavengers is to do them an injustice, for however important this function may be, their service to the body is a far greater one. Most of the material which the blood and lymph streams carry to the body passes through their hands and is stored and it may well be prepared by them for the more highly specialized cell which is to be the ultimate consumer. They are able to carry on in the body true intracellular digestion perhaps by means of a ferment (the makrocytase of Metchnikoff) analogous to the amoebodiastase which Mouton (17) isolated from cultures of amoebae. Recent studies have shown that these cells are capable of carrying on complicated chemical reactions such as the liberation of iron from hemoglobin and they are extremely active in the ingestion and storage of foreign fats, pigment, etc., which come to the tissue via the blood stream. The localization of specialized cells of this class in large numbers along the course of the blood vessels gives them easy access to incoming food-laden fluids and their close relationship to the vascular channels facilitates the disposal of waste which they have winnowed out and liberated from their cytoplasm, or which escapes together with stored food after the destruction of their bodies. Material which has escaped these adventitial cells is seized upon and ingested by the great pyrrhol cells which wander about in the tissue spaces of the body or by specialized "nurse cells" like the makrophages of the testis, in various body organs. Cells of this class receive from the tissue fluids all sorts of "raw" food material which they probably pass, changed and purified by their secretions, to cells less able to perform digestive and selective functions.

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STUDIES ON THE NERVOUS CONTROL OF THE KIDNEY IN RELATION TO DIURESIS AND URINARY SECRETION[1]

I. The Effect of Unilateral Excision of the Adrenal, Section of the Splanchnic Nerve and Section of the Renal Nerves on the Secretion of the Kidney

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One of the authors, Marshall, in collaboration with Davis, found that the complete removal of the adrenals in cats leads to an impairment of kidney function. This is signalized by a rise in the urea content of the blood to about twice its normal value, and a much diminished rate of excretion of urea and creatinine in the adrenalectomized animals compared to the normal, when the kidneys are subjected to increased work by the injection of a mixture of urea, creatinine and sodium chloride. Moreover, these changes may occur with a normal blood pressure and at a time when the cats are in excellent physical condition. It was suggested that these results indicated the secretion of some substance by the adrenals which is necessary for the maintenance of normal renal activity (1). The recent work of Addis and his co-workers (2) strengthens this suggestion. These authors find that the subcutaneous injection of adrenalin in proper dosage increases the "urea excreting activity of the rabbit's kidney." The observations of Marshall and Davis on cats were confirmed for rabbits by these investigators in that they found that "the removal of both suprarenal glands is followed by a depression of the urea excreting activity of the kidney, which is greater than that which follows similar operations in which the suprarenals are not removed." Some time previous to the appearance of the work discussed above, Cow (3) published an article on the regulation of the functional

[1] The data given in this series of papers were presented in abstract before the American Society for Pharmacology and Eperimental Therapeutics, New York, December, 1916. See Proceedings, Journ. Pharm. Exper. Therap., ix, 346, 1917, and before the same society in Baltimore, April, 1919.

activity of the kidneys by means of the adrenals. He claimed both histologically and physiologically to have demonstrated a direct vascular connection between the adrenals and the renal capsule in cats, by which adrenalin could be transported to the kidney directly without first passing through the general circulation. This caused a decrease in the secretion of urine, and hence the adrenals acted as inhibitors of renal secretion so far as the elimination of water was concerned.

These different conceptions of the action of the adrenals on the kidneys are not necessarily opposed to one another, for it might be conceived that they could act at the same time. That is, the products of adrenal secretion accelerate the elimination of urea, but inhibit that of water. In view of this it appeared interesting to us to test out the effect of unilateral removal of the adrenal upon the kidney of that side.

The results obtained indicated at once a definite effect upon the secretion of the kidney on the operated side as compared with the kidney on the normal side. If the adrenals acted as inhibitors for water and accelerators for urea, and if such a control as Cow postulated were functioning, one would expect an increase in the excretion of water and a decrease in the excretion of urea. However, an increase in the excretion of both water and urea was noted on the operated side.

It has been known for many years that section of the splanchnic nerve causes an increased flow of urine. Claude Bernard (4) showed that division of this nerve on one side in the dog increased the secretion of urine on that side. This has been confirmed by Eckhard (5), Knoll (6), Klecki (7), Vogt (8), Grek (9), Rhode and Ellinger (10), and Jungmann and Meyer (11), but is denied by Schwarz (12) and Peyrani (13) who claim a diminished secretion of urine for this procedure.

Since adrenalectomy is always associated with unavoidable injury to the splanchnic which lies immediately behind the gland, the possibility of nerve injury as an explanation for the urinary findings after adrenalectomy seemed very logical. To fortify this new point we chose also to section the nerves which surround the renal artery. This latter procedure does not interfere with the nerve supply of the adrenals and should demonstrate whether one can interpret the findings after adrenalectomy as due wholly to injury to the nerve supply of the kidney.

Another method used in connection with the search for a possible vascular relation between the adrenal and the kidney was the ligation of the adrenal vein at its junction with the vena cava. By this means it was hoped that the vascular connections which Cow describes as coursing from the gland to the capsule of the kidney would show some

influence on the function of the kidney on the operated side, because after ligation of the main exit for the venous blood, the collateral circulation through these vessels would come more into evidence.

METHODS

Dogs were used in all the experiments. As the previous work had been carried out on cats, these animals were first used, but the difficulties encountered in collecting the urine separately from each kidney and in obtaining a sufficient amount for analytical procedures caused them to be discarded. Furthermore, it is probable that the dog's kidney resembles the human more nearly than the cat's. The various operative procedures, excision of the adrenal, section of the splanchnic nerve and section of the renal nerves, were done both immediately before observations were made and also some days or weeks previous. In the latter case, the operations were performed aseptically under ether anesthesia. In the case of excision of the adrenal, the ventral route was chosen. The splanchnic nerve was sectioned just above the adrenal gland, the major splanchnic being always cut, while frequently other strands were also severed. In sectioning the renal nerves the following method was used. The peritoneum was incised and the renal artery and vein exposed. The artery was then freed of all visible nerve strands. This method does not completely denervate the kidney, but is probably freer of objection than painting the artery with phenol. Before making the observations on the secretion of the kidneys, the animals were anesthetized with paraldehyde, 1.7 cc. per kilo by stomach being given. Approximately 20 cc. of water per kilo were given with the paraldehyde. In some cases, where experiments were prolonged, it was found necessary to deepen the anesthesia and a saturated solution of paraldehyde in 0.8 per cent saline was given intravenously. The animals were kept warm by means of an electric hot pad. This procedure produced a very satisfactory anesthesia, and one in which the kidney was apparently but little affected. We were well aware of the danger in using anesthetics in studying renal secretion (14), but believe paraldehyde is freer from objection than the commoner anesthetics which are used. The secretion of urine was usually good even before a diuretic was administered, and the elimination of phenolsulphonepthalein was the same as is shown by unanesthetized animals (15), (16). The ureters were exposed through incisions in the flanks and cannulated with small glass cannulae. All observations were made with the animals in the prone position. This method described by

Quinby and Fitz (17) is more satisfactory than exposing the ureters through the abdomen and making observations in the supine position.

The urine was collected in small graduated cylinders over periods which were usually one hour in duration. The first period was usually one without administering any diuretic, although occasionally 0.8 per cent sodium chloride was injected to start the flow. At the beginning of the second period, diuresis was produced with 10 per cent sodium chloride intravenously which was given in doses of 2 to 4 cc. per kilo. In a few of the earlier experiments, 300 mgms. of diuretin were injected. In some of the experiments the third period consisted in injecting lactose (300 mgms. per kilo) and measuring the amounts eliminated. Phenolsulphonephthalein was given usually at the end of the experiment in the usual dose of 6 mgms. intravenously (15).

The specimens were carefully measured, the specific gravity determined with a small picnometer and analyses made for chlorides, urea and creatinine. Chlorides were determined by the method of McLean and Van Slyke (18), urea by the urease method described by one of us (19), and creatinine by Folin's colorimetric method (20). Phenolsulphonephthalein was determined with a Dubosq colorimeter. Lactose was estimated by the polariscope. At the conclusion of the experiment the kidneys were examined grossly and sections preserved in formaldehyde for histological study. In these experiments urea, creatinine and chlorides were determined in the urine at first because these substances had been previously examined by Marshall and Davis (1) in their work with cats. Later in the course of the investigations other urinary constituents were also determined. The chlorides figures are expressed for the sake of convenience as sodium chloride, although we are well aware that a portion of the chloride of the urine is present in combination with potassium. However, when diuresis was produced by hypertonic sodium chloride, the chlorides excreted were probably mainly in the form of sodium chloride.

RESULTS

Results of ligation of the adrenal vein. The following experiments indicate that ligation of the lumbar vein draining the adrenal has no effect on the secretion of the kidney. If the mechanism postulated by Cow is correct and functions normally, we would expect more epinephrin to be discharged into the kidney on the operated side, and hence expect a smaller flow of urine from that side. In the following summaries of typical experiments under this heading and subsequent ones, the first

column indicates the duration of the periods of collection, the R and L represent the urine from the right and left ureters respectively, and the various urinary constituents are expressed as percentages and also as the total amount in milligrams eliminated during the period.

Experiment 1, Dog MK 8. Female, weight 9.9 kilos, February 9, 1916. On January 29, 1916, operation under ether ligating lumbar vein draining right adrenal.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		per cent	mgm.	per cent	mgm.	per cent	mgm.
10.07–11.07	R	4.5	1.075	3.40	153.0	0.210	9.4	0.04	1.8
	L	4.7	1.075	3.39	159.3	0.182	8.5	0.04	1.9
11.07–12.07	R	23.2	1.032	1.00	232.0	0.033	7.6	1.17	271.0
	L	24.6	1.031	0.97	238.6	0.037	9.1	1.17	286.0
12.07– 1.07	R	17.0	1.040	1.08	183.6	0.038	6.5	1.22	207.0
	L	19.5	1.039	1.03	200.8	0.040	7.8	1.21	235.0

At 1.07, sulphonephthalein was injected and in the next hour the right eliminated 19.0 cc. with 38.5 per cent, and the left 18.5 cc. with 37.2 per cent of the amount injected.

Experiment 2. Dog MK 9. Male, weight 7 kilos, February 11, 1916. On February 5, 1916, lumbar vein draining left adrenal ligated.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		per cent	mgm.	per cent	mgm.	per cent	mgm.
10.50–11.50	R	1.8	1.059	3.64	65.5	0.184	3.3	0.78	14.0
	L	2.4	1.056	3.69	88.6	0.177	4.2	0.71	17.0
11.50–12.50	R	19.3	1.022	1.06	204.0	0.025	4.8	1.47	283.0
	L	20.0	1.020	0.96	198.0	0.024	4.8	1.45	290.0

At 12.50, sulphonephthalein was injected and the right secreted 40 per cent and the left, 42 per cent of the amount injected.

Results of excision of the adrenal. The changes in the secretion of the kidney following removal of the adrenal on one side as compared to the secretion of the kidney on the opposite side may be summarized as follows: The kidney on the operated side secretes more urine usually of a lower specific gravity and lower percentage of urea, constantly of a

lower percentage of creatinine and phthalein and an increased percentage of chlorides. The specific gravity and concentration of urea may be higher on the side with the most urine, as in experiments 3 and 5. During diuresis from injection of hypertonic sodium chloride, the difference in the elimination of water by the two kidneys is augmented, and the specific gravity and percentage of urea, creatinine and phthalein are less on the side with the greater amount of fluid, while the chlorides are still generally increased in percentage. The total quantities of chlorides are greatly augmented, of urea less, and of creatinine and phthalein approximately about the same or only slightly increased on the operated side. The one experiment in which lactose was injected indicates that lactose resembles creatinine and phthalein. These changes are the same whether the observations are made immediately after removal of the adrenal or some weeks later.

Experiment 3. Dog MK 14. Male, weight 10.5 kilos, March 17, 1916. Left adrenal removed one hour and a half before experiment was begun.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
12.06–12.46	R	1.8	1.038	1.68	30.2	0.271	4.9		
	L	2.2	1.056	3.60	79.2	0.228	5.0		
12.46– 1.46	R	4.0	1.052	1.86	74.4	0.164	6.5	0.43	17.2
	L	22.0	1.027	1.06	233.2	0.031	6.8	1.13	248.6

At 1.46 sulphonephthalein injected and in next hour, right eliminated 7.0 cc. containing 36.8 per cent, and left, 24.0 cc., containing 39.1 per cent of amount injected.

Experiment 4. Dog MK 17. Male, weight 9.4 kilos, March 22, 1916. Left adrenal removed about one hour and a half before commencing experiment; 10 per cent saline given before first period.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
1.00–1.45	R	6.5	1.025	0.82	53.3	0.076	4.9	1.31	85.1
	L	32.5	1.020	0.62	201.5	0.021	6.8	1.61	523.2
1.45–2.30	R	9.2	1.028	1.26	115.9	0.078	7.2	1.65	151.8
	L	27.0	1.021	0.81	218.7	0.027	7.3	1.77	477.9

At 2.30, sulphonephthalein injected and in next hour, right eliminated 15.5 cc. with 41.0 per cent, and left, 36.5 cc. with 44.3 per cent of amount injected.

Experiment 5. Dog MK 15. Male, weight 13.5 kilos, March 25, 1916. Left adrenal removed March 18, 1916.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
12.00–1.00	R	2.6	1.059	2.04	53.0	0.326	8.5	0.14	3.6
	L	5.2	1.057	3.90	203.0	0.176	9.1	0.44	22.9
1.00–2.00	R	7.0	1.040	2.29	160.3	0.111	7.8	0.87	60.9
	L	20.5	1.026	1.23	252.2	0.041	8.4	1.21	248.0

At 2.00, 4 grams lactose in about 20 cc. water injected intravenously. In the next hour, right eliminated 13.7 cc., containing 1.20 grams, and left, 23.0 cc., containing 1.24 grams. In next hour, sulphonephthalein was excreted 44.3 per cent on the right, and 44.0 per cent on the left.

Experiment 6. Dog MK 21. Male, weight 11.2 kilos, March 28, 1916. Right adrenal removed on November 5, 1915.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
3.00–4.00	R	10.1	1.045	3.87	390.9	0.079	7.8	0.80	80.8
	L	3.9	1.054	5.70	222.3	0.185	7.2	0.16	6.2
4.00–5.00	R	62.5	1.020	0.99	618.8	0.012	7.5	1.43	893.8
	L	17.0	1.028	2.17	368.9	0.040	6.8	1.33	226.1

At 5.00 sulphonephthalein injected, right excreted 33.0 cc. with 47.0 per cent, and left, 7.0 cc. with 41.0 per cent of amount injected.

Results of section of the splanchnic. The changes after unilateral section of the splanchnic nerve are seen from the data in the following experiments to be identical with those following unilateral excision of the adrenal. The kidney on the side of the section eliminates more urine of a lower (or higher) specific gravity, and there is decreased (or increased) percentage of urea, decreased percentage of creatinine and phthalein, and increased percentage of chlorides. During sodium chloride diuresis, the urine is greater in amount and always of a lower specific gravity, there is a decreased percentage of urea, creatinine and phthalein, and an increased percentage of chlorides. The absolute amount of chlorides eliminated is greater on the operated side, of urea greater but in a less degree than chlorides, and of creatinine and phthalein only slightly greater or the same. The few experiments with lactose indicate that it resembles creatinine and phthalein in its excretion.

Experiment 7. Dog MK 23. Female, weight 5.70 kilos, April 6, 1916. Left splanchnic nerve major and minor sectioned two hours before commencing observations.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		per cent	mgm.	per cent	mgm.	per cent	mgm.
12.22–1.25	R	2.0	1.050	2.04	40.8	0.250	5.0	0.44	8.8
	L	4.6	1.037	2.94	135.2	0.114	5.2	0.98	45.0
1.25–2.25	R	18.0	1.021	1.22	219.6	0.028	5.0	1.31	235.8
	L	34.0	1.015	0.68	231.2	0.014	4.9	1.33	452.2

At 2.25, sulphonephthalein was injected, and in the next hour, right eliminated 21.0 cc. with 38.5 per cent, and left, 58.0 cc. with 39.5 per cent of amount injected.

Experiment 8. Dog MK 22. Male, weight 7.4 kilos, April 20, 1916. Left splanchnic sectioned April 1, 1916.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE	
		cc.		per cent	mgm.	per cent	mgm.
12.45–1.50	R	1.7	1.034	6.06	103.0	0.364	6.2
	L	3.2	1.037	6.99	223.7	0.210	6.7
1.50–2.50	R	9.5	1.024	2.26	214.7	0.064	6.1
	L	27.5	1.016	1.00	275.0	0.022	6.1

At 2.50, 2.2 grams lactose injected intravenously. During the next hour 1.05 grams in 13.8 cc. urine were eliminated by the right kidney, and 0.92 gram in 27.6 cc. urine by the left; 3.50–4.50, right eliminated 41.5 and left, 45.0 per cent of injected phenolsulphonephthalein.

Experiment 9. Dog MK 32. Female, weight 8.0 kilos, July 18, 1916. Left splanchnic sectioned on May 19, 1916.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		per cent	mgm.	per cent	mgm.	per cent	mgm.
1.35–2.35	R	2.05	1.055	3.10	63.6	0.218	4.5		
	L	2.50	1.053	2.42	60.5	0.214	5.4		
2.35–3.35	R	11.5	1.024	1.10	126.5	0.050	5.8	1.21	139.2
	L	21.0	1.019	0.73	153.3	0.029	6.1	1.33	270.3
3.35–4.35	R	13.8		0.84	115.9	0.042	5.8	1.17	161.5
	L	21.6		0.65	140.4	0.029	6.2	1.28	276.5

At 3.35, 2.4 grams lactose were given intravenously; in the period 3.35–4.35, 716 mgm. were eliminated by right kidney and 850 mgm. by the left. At 4.35, sulphonephthalein was injected and during the next hour, right eliminated 35 per cent, and left, 37 per cent of amount injected.

Experiment 10. Dog MK 27. Male, weight 8.6 kilos, November 17, 1916. Left splanchnic sectioned and piece removed on April 26, 1916.

TIME		URINE	UREA		CREATININE		SODIUM CHLORIDE	
		cc.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
11.13–12.13	R	2.8	5.82	163.0	0.217	6.0	0.58	16.2
	L	4.0	4.47	178.8	0.143	5.7	0.91	36.4
12.13– 1.13	R	10.8	2.62	283.0	0.058	6.3		
	L	23.0	1.48	340.4	0.027	6.1		

At 1.13, sulphonephthalein given and in next hour right eliminated 9.3 cc. with 33.0 per cent, and left, 27.5 cc. with 34.0 per cent of amount injected.

Results of section of the renal nerves. The changes incident to sectioning the renal nerves on one side are, as far as can be judged, by the experiments carried out, identical with those of sectioning the splanchnic or removing the adrenal gland. The following summaries of experiments indicate this.

Experiment 11. Dog MK 33. Female, weight 7.0 kilos, June 7, 1916. Renal nerves on left side sectioned one-half hour before commencing observations.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
11.45–12.45	R	3.25	1.053	1.26	41.0	0.069	2.2	Trace	
	L	3.80	1.054	1.20	45.6	0.066	2.5	Trace	
12.45– 1.45	R	5.0	1.048	1.47	73.5	0.067	3.3	0.80	40.0
	L	8.0	1.041	1.17	99.5	0.040	3.4	1.03	87.6

At 1.45, 2.1 grams lactose injected intravenously. In next hour, right eliminated 4.3 cc. containing 670 mgm., and left, 8.0 cc. containing 736 mgm. At 2.45, sulphonephthalein was injected, right eliminated 5.5 cc. with 47.6 per cent, and left, 12.2 cc. with 46.2 per cent of amount injected.

Experiment 12. Dog MK 34. Male, weight 4.8 kilos, June 13, 1916. Renal nerves on left side sectioned one hour before commencing observations.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
11.15–12.45	R	2.35	1.079	3.84	90.2	0.167	3.9	0.04	0.9
	L	3.85	1.072	3.09	119.0	0.108	4.2	0.18	6.9
12.45– 1.45	R	5.2	1.036	2.22	115.4	0.043	2.2	1.40	72.8
	L	25.0	1.022	0.74	185.0	0.011	2.8	1.35	337.5
1.45– 2.45	R	5.8		0.72	41.8	0.038	2.2	1.26	73.1
	L	26.0		0.47	122.2	0.009	2.3	1.39	361.4

In the third period, 1.44 grams of lactose were injected intravenously. At 2.45, sulphonephthalein was given, and in next hour right secreted 2.3 cc. containing 34.3 per cent, and left, 11.6 cc. containing 35.8 per cent of amount injected.

Experiment 13. Dog MK 35. Male, weight 6.2 kilos, June 19, 1916. Renal nerves on left side sectioned June 14, 1916.

TIME		URINE	SPECIFIC GRAVITY	UREA		CREATININE		SODIUM CHLORIDE	
		cc.		*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
10.55–11.45	R	5.15	1.031	1.95	100.4	0.115	5.9	0.86	44.3
	L	11.00	1.024	2.01	221.1	0.053	5.8	1.01	111.1
12.40– 1.40	R	8.3		1.67	138.6			1.21	100.4
	L	25.5		0.59	150.5			1.45	369.8

Experiment 14. Dog MK 84. Male, 4.5 kilos, January 29, 1917. Left renal nerves sectioned on January 3, 1917.

TIME		URINE	UREA		CREATININE		SODIUM CHLORIDE	
		cc.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
11.45–12.45	R	1.0	3.69	36.9	0.160	1.60		
	L	1.8	3.75	57.5	0.091	1.65		
12.45– 1.45	R	2.0	1.76	35.2	0.095	1.90	0.34	6.8
	L	4.5	1.43	64.5	0.042	1.90	0.62	27.9

At 1.45, sulphonephthalein was injected and in next hour, right eliminated 2.5 cc. with 29.3 per cent, and left, 6.2 cc. with 29.7 per cent of amount injected.

DISCUSSION

As stated above, the effect upon the secretion of the kidney after unilateral extirpation of the adrenal consists in the secretion of a greater volume of urine of lower specific gravity, a higher percentage and absolute amount of chlorides, a decreased percentage but greater absolute amount of urea, and a decreased percentage but approximately the same amounts of creatinine and phenolsulphonephthalein. We are not concerned here with the question as to whether the creatinine and sulphonephthalein are secreted in exactly the same amounts by the two kidneys. The figures indicate sometimes a slight increase, sometimes a slight decrease, and sometimes no change on the side with the greater flow. The important point is that no unmistakable and constant change occurs in the secretion of creatinine and phenolsulphonephthalein as is seen with water, urea and chlorides.

These characteristic changes can be produced equally well by section of the splanchnic nerve or by section of the renal nerves. In fact, the effects of these three procedures cannot be distinguished from one another by examining the urines secreted by each kidney. In removing the adrenal gland, injury to the fibers of the splanchnic nerve is unavoidable. It would appear, therefore, unnecessary to postulate any direct connection of the adrenal with the kidney of the same side to explain the phenomena observed; that these phenomena can be reproduced by nerve sectioning where the nerve supply of the adrenal gland is undisturbed is shown by the experiments on cutting the renal nerves.

The relation between the adrenals and the kidney as postulated by Cow (3) does not appear to function normally in the dog. This is further shown by the experiments on ligating the lumbar vein of one gland. No change in the urine on that side occurs. However, the inter-relations of the adrenals and kidneys as shown by Marshall and Davis (1) and signalized by a decreased functional capacity of the kidneys after the total ablation of the glands, and that indicated by Addis and his co-workers (2) by showing an effect of adrenalin on the urea excreting function of the kidneys are in no wise affected or invalidated by these experiments. In these cases, the effects can be produced through the general circulation, and not by local connections of the adrenal and kidney.

Regarding the mechanism of the production of the changes in the secretion of the kidney after splanchnotomy, the usual explanation given is an effect on the blood flow through the kidney, although some

investigators are inclined to attribute it to a specific secretory effect of the nerve. In the following paper, we are inclined to believe that we have accumulated positive evidence that the first of these explanations is sufficient.

The behavior of the different urinary constituents in relation to the different volumes of urine secreted by each kidney in these experiments is similar, in some respects, to that observed in similar experiments and also in experiments of a somewhat different nature. Knoll (6) examined the urine in dogs after cutting one splanchnic, and found the urine of the operated side of a lower specific gravity, lower percentage of solids and urea (determined by Liebig's method), while the total elimination of solids and urea was greater. Grek (9) noted an increase in the absolute as well as percentage amount of chlorides after unilateral splanchnotomy in the dog. This was confirmed by Jungmann and Meyer (11). In a short preliminary note Rhode and Ellinger (10) state that after unilateral section of the splanchnic or renal nerves, the urine of the operated side contains a smaller percentage of solids (as determined by specific gravity and freezing point) but a greater absolute amount. The acidity to phenolphthalein was less, but the total amount of acid eliminated was greater. These changes were observed in animals subjected to operations weeks or months previously. No details are given in their preliminary note and the detailed communication promised has not come to our notice.

The increased elimination of urine by the kidney on the operated side in these experiments can be considered in the nature of a diuresis as compared to the secretion of the organ of the normal side. Certain changes in the constituents of the urine are characteristic of diuretic urine in general. V. Schröeder (21), studying caffeine diuresis in the rabbit, found the total solids and nitrogen of the urine increased but not to the same extent as the water. Thompson (22) noted the same for nitrogen and urea during sodium chloride diuresis. Katsuyama (23) found caffeine diuresis in the rabbit accompanied by an increase in the alkali chlorides, but to a greater extent in the case of the sodium than the potassium. Later (24), he noted the same for urea and diuretin diureses. Loewi's (25) studies on diuretics, mainly caffeine and sodium nitrate injected into dogs, indicated during diuresis an increase in the chlorides, urea (total nitrogen), sugar in hyperglycemia, and injected phosphates, but no change in the elimination of the sugar from phlorhizin diabetes or the phosphates formed in metabolism. However, Bock (26) working with rabbits found that injection of solutions of

glucose, sodium chloride and sodium sulphate, and purine derivatives produced a diuresis with increase of the phosphates, while a diuresis by the administration of water by mouth caused no increase in the phosphate elimination. Baetzner (27), on the other hand, has failed to confirm Bock's observations that water diuresis causes no increase in the elimination of phosphates in rabbits, but found a very marked increase. Rowntree and Geraghty (15) studied the influence of various diuretics on the elimination of phenolsulphonephthalein in cats. They came to the conclusion that

> under the conditions of our experiments it was found that those diuretics which are known to exert some stimulating influence on the activity of the secreting cells, or those diuretics in connection with which evidence is at hand indicating a stimulating action on the secreting cells (caffeine, urea, dextrose, phlorhizin, calomel), slightly increase the phthalein output, whereas those diuretics which act entirely by changes in osmotic tension or by changes in blood-pressure, etc. (hypertonic sodium chloride solution, potassium nitrate and digitalis), apparently have little or no effect on its excretion.

To sum up, then concerning the effect of diuresis on the elimination of urinary constituents, the total solids, total nitrogen, urea and chlorides are increased while the phosphates are probably increased to a lesser extent. The percentage of chlorides may even rise in the diuretic urine, while the other constituents are decreased in percentage at least during the height of the diuresis. The phenolsulphonephthalein excretion is not influenced by diuresis produced by sodium chloride. Cushny (28), in his recent monograph, calls particular attention to the difference in behavior of substances during diuresis. According to him, all the substances of the urine are increased in amount during diuresis. The "no-threshold" substances—urea, phosphates, sulphates—are invariably reduced in percentage but slightly increased in absolute amount, while the "threshold" substances—sodium chloride—are often reduced in percentage, but may actually rise in some circumstances.

The changes which we have observed, therefore, are similar to those characteristic of an increased flow of urine, the percentage of chlorides rising while that of urea, creatinine and sulphonephthalein falls. The total amount of chlorides and urea is markedly increased, while the creatinine and phthalein are approximately unchanged. The behavior of creatinine in diuresis has not been studied, but it is known to be more or less independent of the volume of urine excreted.[2] Similar changes

[2] Our experiments reported in this paper would tend to show that creatinine may be independent of diuresis. However, they were not performed with this object. More careful experiments are being carried out on this point.

in the behavior of various urinary constituents have been observed where the secretion of one kidney was lessened by opposing an obstruction or resistance to the flow. It was shown that water and chloride decrease greatly in amount on the side with the lesser flow, while urea, phosphates, sulphates and indigo-carmine are much less decreased. Moreover, the last named constituents are present in higher percentage while the chloride may be present in higher percentage on the side with the smaller secretion (28). The behavior of creatinine and phthalein under these conditions has not been examined.

The changes in the urine after section of the splanchnic, extirpation of the adrenal or section of the renal nerves appear to persist for months after the operation. This was also noted for the section of the splanchnic and renal nerves by Rhode and Ellinger (10). On the other hand Quinby (29), in experiments on removing the dog's kidney and re-implanting it, found the changes in the urine of the operated side to disappear after a period of 10 to 14 days. In one experiment on the section of the renal nerves, observations made 20 days later indicated that the secretion of each kidney was identical, but this experiment is opposed by many others, particularly on section of the splanchnic or removal of the adrenal. Further work is necessary to explain the apparent discrepancy with Quinby's work, but it must be remembered that the procedures employed are not identical.

The changes in the various constituents of the urine after these experimental procedures, which are similar in many ways to the changes induced by other experimental methods of causing an increased or decreased flow of urine from one kidney, and their bearing on the theories of urinary secretion, will be discussed in a subsequent communication.

SUMMARY

The changes produced in the secretion of one kidney by unilateral removal of the adrenal can be duplicated by sectioning the splanchnic nerve on one side, and also by section of the nerves on the renal artery and vein. It is not necessary to postulate any direct functional vascular connections between the adrenal and the kidney, as has been claimed by Cow, to explain these changes. Unilateral removal of the adrenal appears to affect the kidney of the same side only in so far as the nerves going to the kidney have been injured. This work, however, in no way invalidates the conclusion that complete removal of the adrenals depresses the function of the kidneys.

After the experimental procedures employed in this work,—unilateral removal of the adrenal, section of the splanchnic or section of the renal

nerves—the kidney on the operated side secretes in general a more dilute urine containing a greater percentage of chlorides but a smaller percentage of urea, creatinine, lactose and phenolsulphonephthalein. This is always the feature during diuresis produced by sodium chloride, but during a normal flow the urea percentage may be higher on the side with the greater amount of urine. The total amount of water, chlorides and urea is greater on the operated side, while but little or no change is noticed in the total amount of creatinine and phthalein eliminated on the two sides. The similarity of these changes to those occurring during diuresis and in the lessened flow of urine produced by partial obstruction of the ureter has been discussed.

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STUDIES ON THE NERVOUS CONTROL OF THE KIDNEY IN RELATION TO DIURESIS AND URINARY SECRETION

II. A Comparison of the Changes Caused by Unilateral Splanchnotomy With Those Caused by Unilateral Compression of the Renal Artery

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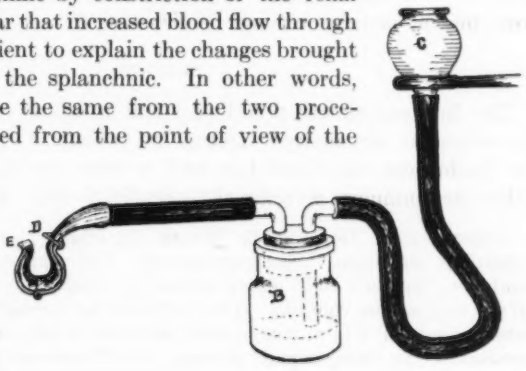
In the preceding paper, certain changes occurring in the urine after section of the splanchnic nerve on one side have been described. Many investigators are inclined to attribute any effects of the nerves upon the secretion of the kidney as due to vasomotor influences (1). The question, however, of true secretory action of the splanchnic or vagus upon the renal cells is an old one, but several recent investigations have been concerned with this problem. Asher and Pearce (2) claimed to have demonstrated secretory fibers in the vagus, but later Pearce and Carter (3) failed to confirm the earlier work, finding no change in the oxygen consumption of the kidney when secretory action was supposed to take place. Jungmann and Meyer (4) found an increase in the chlorides both in percentage and absolute amount following puncture of the floor of the fourth ventricle and that this was abolished after section of the splanchnic. Rhode and Ellinger (5) found that the changes after unilateral section of the renal nerves persisted for weeks or months after the operation. These changes they state to consist in the secretion of a larger volume of urine, of a lower percentage content in solids, and acidity to phenolphthalein, but a greater total amount of solids and acids. These last two investigators conclude that the splanchnic exerts a specific inhibitory-secretory action on the renal cells, which cannot be explained by vasomotor changes. Jost (6) has also advanced evidence that stimulation of the splanchnic can cause a diminution of the urine flow aside from the vasomotor effect. The evidence of these investigators, however, is not conclusive and does not appear to be generally accepted (1). Numerous investigations have shown that an animal can survive with a kidney which is completely deprived of its

nerves, and Lohenhoffer and Quinby have shown that a transplanted or re-implanted kidney suffices for life and also shows a perfectly normal function (7). In the last year, Addis and his co-workers (8) have again advanced the idea of specific nervous control of the kidney to explain their results of the increase in the rate of urea excretion caused by adrenalin and the decrease caused by pituitrin. The changes, which we have described in the preceding paper of this series as resulting from the section of the splanchnic nerve, have been shown to resemble the change occurring in the urine in general when the secretion is markedly increased or decreased. The similarity to forms of diuresis and in partial obstruction of the ureter has been pointed out by Cushny (9), and has been discussed in our previous communication. It would appear then on *a priori* grounds that a specific secretory action of the splanchnic nerve need not be invoked to explain these changes.

The splanchnic nerve is known to contain the vasomotor fibers for the renal vessels, and its section would produce a vasodilatation in the kidney with a corresponding increased amount of blood flowing through the organ. In fact, Burton-Opitz (10) has demonstrated by the use of his "stromuhr" that stimulation of the splanchnic causes a diminished flow of blood through the kidney while section of the same nerve causes an increased blood flow through the organ. Moreover, the splanchnic nerve of each side supplies only the renal vessels of that side (11). Can the changes in the secretion of the kidney after section of the splanchnic be attributed entirely to the increased blood flow through the organ? The blood flow through the kidney can be conveniently diminished by partial constriction of the renal artery. A comparison of the changes produced by section of the splanchnic with those produced by decreasing blood flow suggested itself as an aid in determining the cause of these changes. The changes following partial constriction of the renal artery in rabbits have been examined by Yagi and Kuroda (12). They found with diminished blood flow, after producing diuresis by injection of salines, a decreased amount of urine, chlorides, urea and sulphates. When a mixture of sodium chloride and sodium sulphate was injected as a diuretic, the reduction in chlorides was much greater than the fluid on the constricted side, that is, the percentage of chlorides was greater on the side of greater flow. When a mixture of urea and sodium chloride was used to produce diuresis, the water was more affected than chlorides. In both cases the urea was less affected than the water or chlorides, and the sulphates still less so. Thus, we see that the changes produced here are similar to those caused by section of the splanchnic

when the kidneys of the sides secreting the greater quantity of urine are compared. However, the elimination of creatinine or phenolsulphonephthalein was not examined by these authors, their work was carried out on rabbits, and the secretion was only compared under the influence of a mixture of diuretics. Since our previous work on the section of the splanchnic had been carried out on dogs, and the changes in the elimination of chlorides, urea, creatinine and phenolsulphonephthalein extensively studied both during normal secretion and in diuresis produced by sodium chloride, we considered it important to re-investigate the effect of partial occlusion of the renal artery. Should it be possible to produce changes of an opposite nature to those obtained by sectioning the splanchnic by constriction of the renal artery, it would appear that increased blood flow through the kidney was sufficient to explain the changes brought about by section of the splanchnic. In other words, the changes might be the same from the two procedures when considered from the point of view of the secretion of different amounts of urine, in one case one kidney eliminating more urine by virtue of the splanchnic being sectioned and in the other the organ on the normal side secreting more on account of decreased blood flow on the other side. If the changes occasioned by nerve section could be annulled by pressure on the artery, the value of the argument would be increased.



PRESSURE CUFF

Fig. 1

The methods employed were those described in the previous communication (13). The renal artery was exposed by an incision through the flank, and care was taken to disturb the nerves as little as possible, but the results show that some nerves were generally injured. We wished to adjust the pressure carefully so as to be able to study the effect of pressure on the renal artery after section of the splanchnic nerve on the same side. The apparatus used for exerting the pressure was a small rubber cuff, enclosed in an aluminium band. The cuff could be

filled with water and pressure exerted by raising a column of mercury. This apparatus we have called the "pressure cuff." It is illustrated in figure 1. *A* is a flattened tube of thin rubber dam which is covered with thin silk gauze or bolting silk and attached to a piece of rubber tubing. *B* is a small glass bottle, and *C* a thistle tube. *A* is filled with water and surrounded by the hinged aluminium band, then slipped about the artery and fastened by slipping the wire clasp *D* over the small projecting piece of metal, *E*. The tube connected to *A* is then passed through a small incision made conveniently near the kidney, and passed to the outside where *B* which has previously been filled with water and *C* are attached. *C* is then filled with mercury. Various degrees of pressure can be exerted on the renal artery by raising or lowering the mercury column by elevating *C*.

RESULTS

The first experiment described is one in which an attempt was made to neutralize the changes induced by section of the splanchnic nerve by diminishing the blood flow with pressure on the renal artery. The following summary presents the essential details and tabulated results.

Experiment 1. Dog MK 81. Weight 7.1 kilos. December 22, 1916. Left splanchnic sectioned. Ureters cannulated. Left renal artery exposed through flank and "pressure cuff" placed around it. Diuresis produced by injection of 10 per cent sodium chloride. Urine collected for one-half hour periods without pressure on renal artery, then pressure adjusted so that each kidney secreted approximately the same amounts of urine. At 4.38 pressure had been satisfactorily adjusted; at 5.41 pressure was completely removed from left renal artery.

	TIME		URINE	UREA		CREATININE		SODIUM CHLORIDE	
			cc.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
I	3.25–3.55	R	13.0	0.55	72.0	0.012	1.60	1.20	156.2
		L	26.5	0.34	90.1	0.006	1.65	1.23	325.9
II	3.55–4.25	R	8.6	0.49	42.5	0.017	1.50	1.18	101.3
		L	30.5	0.28	85.4	0.004	1.47	1.29	393.5
III	4.38–5.08	R	6.75	0.87	59.2	0.020	1.47	1.00	67.5
		L	7.75	0.67	51.9	0.017	1.35	1.16	90.0
IV	5.10–5.40	R	8.00	0.88	70.5	0.020	1.63	0.81	65.0
		L	11.00	0.53	58.5	0.012	1.38	1.13	125.0
V	5.43–6.13	R	5.7	0.75	42.7	0.026	1.52	0.57	32.5
		L	22.2	0.38	76.5	0.007	1.57	1.01	225.0

In the first two periods the urine from the operated side exhibits all the changes described as characteristic of splanchnic section. In the third period, the pressure adjusted has been just about enough to cause the two kidneys to secrete about equal amounts of urine, and the figures are not far from those obtained from an experiment on a normal animal. The correspondence to the normal is not exact but much better than one might expect when we realize that diminishing the blood flow by constricting the renal artery is not at all identical with nature's method of increasing it by vasodilatation in the various branches of the renal artery. In period IV, the pressure is not as effective or has decreased and the correspondence with the normal is not as close. That the kidney has not been injured by this slight pressure on the artery is probable when we see in period V, where the pressure is removed, a quick return to the condition of periods I and II or at the commencement of the experiment.

The following summaries are typical of other experiments in which the blood flow through the kidney was diminished by pressure on the renal artery.

Experiment 2. Dog MK 98. Weight 7.80 kilos. February 5, 1917. "Pressure cuff" placed on left renal artery, and ureters cannulated. Diuresis produced by injection of 10 per cent sodium chloride and physiological saline. After operation, animal was allowed to recover for about one hour, then collection of urine from each kidney commenced. At 1.18 pressure applied to left renal artery, and adjusted so that the left kidney was secreting about one-half as fast as the right. At 2.22 pressure lessened slightly. At 3.19 pressure removed.

TIME		URINE	UREA		CREATININE		SODIUM CHLORIDE	
		cc.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
12.15–12.45	R	3.0	1.67	50.2	0.033	0.99	1.16	35.0
	L	3.0	1.51	45.2	0.032	0.96	1.24	37.4
12.48– 1.18	R	12.1	0.87	106.2	0.011	1.38	1.41	170.7
	L	11.9	0.78	93.6	0.011	1.25	1.45	173.4
1.52– 2.22	R	12.7	0.51	65.3	0.007	0.99	1.56	200.7
	L	7.4	0.61	44.8	0.012	0.92	1.27	93.0
2.48– 3.18	R	10.5	0.58	60.9	0.009	1.02	1.24	130.8
	L	7.9	0.65	51.8	0.012	0.99	1.34	106.2
3.27– 3.57	R	6.8	0.55	37.8	0.011	0.75	1.12	77.7
	L	6.2	0.57	35.7	0.011	0.68	1.25	77.7

Experiment 3. Dog MK 102. Weight 7.80 kilos. February 13, 1917. "Pressure cuff" placed on left renal artery, and ureters cannulated. At 1.37 slight pressure on left renal artery. At 2.40–3.30 pressure very much increased on left renal artery, and adjusted so that the right kidney was secreting about ten times

as much as the left. At 4.08 pressure completely removed. At 11.23–12.00, intravenous infusion of 50 cc. of a 3 per cent sodium chloride, 3 per cent urea and 0.3 per cent creatinine solution. At 3.37, infusion of 15 cc. more of above mixture.

TIME		URINE	UREA		CREATININE		SODIUM CHLORIDE	
		cc.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
12.23–12.53	R	11.4	1.28	145.3	0.162	18.5	0.93	106.7
	L	14.0	1.07	150.8	0.127	17.8	0.94	132.0
1.37– 2.07	R	17.8	0.84	150.1	0.092	16.4	1.08	193.7
	L	15.3	0.86	131.9	0.101	15.5	1.26	193.2
2.07– 2.37	R	10.0	1.01	100.8	0.108	10.8	1.16	116.0
	L	10.0	1.02	101.7	0.105	10.5	1.20	120.0
3.37– 4.07	R	12.5	1.32	164.7	0.086	10.8	1.28	160.0
	L	1.5	2.92	43.8	0.740	11.1	1.73	26.0
4.14– 4.44	R	7.8	0.45	35.3	0.072	5.6	1.39	108.7
	L	11.6	0.80	93.5	0.047	5.4	1.25	145.0

Experiment 4. Dog MK 99. Weight 6.4 kilos. February 7, 1917. At 11.15, "pressure cuff" placed on left renal artery. Ureters cannulated. Between 1.30 and 2.30, pressure adjusted on left artery. At 3.35 pressure considerably increased. At 4.10 pressure removed.

TIME		URINE	UREA		CREATININE		SODIUM CHLORIDE	
		cc.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
12.20–12.50	R	4.25	2.33	99.0	0.047	2.01	1.80	76.5
	L	5.70	1.80	102.6	0.035	2.00	1.66	94.4
12.50– 1.20	R	8.0	0.81	64.4	0.018	1.46	1.65	132.0
	L	12.5	0.63	79.7	0.012	1.56	1.60	200.4
2.30– 3.00	R	19.2	0.49	94.2	0.008	1.59	1.54	296.8
	L	9.4	0.64	60.0	0.015	1.38	1.57	148.0
3.05– 3.35	R	18.5	0.50	93.0	0.008	1.51	1.55	287.2
	L	12.6	0.66	83.4	0.011	1.42	1.60	200.8
3.40– 4.10	R	20.2	0.44	88.5	0.007	1.44	1.59	322.8
	L	8.2	0.76	62.4	0.016	1.34	1.48	122.0
4.55– 5.25	R	14.2	0.53	76.5	0.011	1.55	1.76	250.5
	L	13.3	0.57	77.0	0.011	1.47	1.74	232.5

DISCUSSION

The changes caused in the secretion of the kidney by diminishing the blood flow by compression of the renal artery are exactly in the opposite direction to those caused by section of the splanchnic nerve. Water is diminished, chlorides are frequently diminished in percentage as well as absolute amount, while urea and creatinine are increased in percentage but diminished in absolute amount. The creatinine is excreted in nearly the same quantities by the two kidneys, although in general the one with the diminished blood flows tends to excrete slightly less. It might be mentioned again that decreasing the blood flow by constricting the renal artery may not be exactly comparable to decreasing it by a mild vasoconstriction in the body, and moreover, we have found that if the blood flow is decreased to a great extent, all substances excreted fall markedly in absolute amount on the affected side. A certain blood flow is apparently necessary to furnish enough oxygen for nutrition of the renal cells.

The following table which is collected from experiments reported in the preceding article and those given in this paper indicate the identity of the changes when the blood flow is decreased and when the splanchnic is sectioned. In the case of the experiments in which nerves are severed,

Comparison of changes after section of splanchnic and after compression of renal artery

PROCEDURE		WATER	CHLORIDE	UREA	CREATININE	DOG NUMBER
Compression on renal artery	R	171	217	146	107	MK 98
	L	100	100	100	100	
Section of splanchnic	R	100	100	100	100	MK 32
	L	182	200	121	105	
Compression on renal artery	R	147	143	111	105	MK 99
	L	100	100	100	100	
Section of splanchnic	R	100	100	100	100	MK 32
	L	156	171	121	107	
Compression on renal artery	R	250	263	143	107	MK 99
	L	100	100	100	100	
Removal of left adrenal	R	100	100	100	100	MK 15
	L	293	407	157	107	

the secretion of the kidney of the normal side is represented as 100, and that of the other compared to this. In expressing the results of experiments on diminished blood flow, the secretion of the kidney with the lesser flow is taken as 100 and the one on the normal side compared to this. Data are selected not at random, but to obtain as close a correspondence as possible for it is our desire to show only that the changes characteristic of section of the splanchnic can be reproduced by a procedure which affects the blood flow.

It appears then that there is little doubt that the changes which have been described as characteristic of sectioning the splanchnic or renal nerves can be entirely reproduced by compressing the renal artery. This, of course, would cause a diminished pressure in the kidney as well as a diminished blood flow and smaller amount of oxygen to be utilized. However, increasing the blood flow would also cause an increased pressure and an increased amount of oxygen. Richards and Plant (14), in their extremely important experiments on the perfusion of the kidney by a method free from the objections coincident to so many perfusion experiments, found that urine flow depended more on the rate of blood flow than blood pressure in the kidney. It is conceivable that the effects of increased and decreased blood flow depend entirely on the carrying of more or less oxygen to the kidney, but this is unlikely as long as the amount of oxygen is sufficient for the needs of the cells. Barcroft and Straub (15) found that blood flow might frequently change without any change in the oxygen consumption. It seems reasonable then to ascribe the effects of compression of the renal artery (provided it is not carried to too great an extreme, where decreased oxygen supply undoubtedly enters as a factor) to diminished blood flow. The changes, therefore, caused by sectioning the splanchnic or renal nerves can be explained by the increased blood flow known to occur under these conditions.

The fact that the changes characteristic of splanchnotomy persist for weeks and months after the operation has been advanced as an argument that mere vasodilatation cannot explain the changes. The vessels by this time would be supposed to have regained their tone. But as Bayliss (16) has pointed out during the observations, vasoconstrictor impulses, partly reflex, are being sent to the vessels of the kidney on the uninjured side, while they are absent on the side with sectioned nerves. Again, our experiments with nicotine would tend to support this as the differences in secretion of the two kidneys, even after the splanchnic has been sectioned for months, can be caused to disappear

after administration of nicotine. This is discussed more fully in a subsequent communication of this series.

It appears, therefore, that the burden of proof still rests with those who would assign a specific secretory-inhibitory action to the splanchnic nerve aside from the changes which it causes by being the chief vasomotor nerve to the kidney. As long as the changes produced can be explained entirely as vasomotor phenomena, it is unnecessary to invoke a specific secretory action for the nerve.

SUMMARY

The changes caused in the secretion of the kidneys after section of the splanchnic nerve on one side are in all respects examined similar to those caused by changes in blood flow through the kidney. The effect of section of the splanchnic is an increased flow through the kidney which is responsible for the changes in secretion. It appears unnecessary at present to assign a specific secretory function to the nerve to explain these changes.

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STUDIES ON THE NERVOUS CONTROL OF THE KIDNEY IN RELATION TO DIURESIS AND URINARY SECRETION

III. The Effect of Nicotine on the Secretion of the two Kidneys after Unilateral Section of the Splanchnic Nerve

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It is well known that nicotine, after a brief period of stimulation, causes a paralysis of sympathetic ganglia. It appeared probable that the injection of nicotine into an animal, in which unilateral section of the splanchnic had been performed, might amount to the section of the nerve on the other side. The effects, then, of unilateral splanchnotomy should be abolished by the injection of nicotine as far as the difference in the secretion of the two kidneys is concerned.

The methods employed were similar to those described previously. Blood pressure, urine flow and kidney volume were recorded in some of the experiments. A modified type of the Roy oncometer was used.

The following protocol (exper. 1) is illustrative of the general type of procedure employed. The effect of section of the splanchnic in producing a relative increase in the amount of urine on the operated side, as well as the effect of nicotine injected intravenously, of promptly causing the difference in fluid secretion of the two kidneys to disappear is clearly shown. Before section of the splanchnic nerve, the ratio of the amount of urine secreted by each kidney is nearly unity; after section of the nerve, the left kidney secretes about three times as much as the right; the intravenous injection of nicotine causes the two kidneys to again return to the normal ratio and secrete the same amounts of urine.

One of the most characteristic changes in the composition of the urine after section of the splanchnic nerve is a relative, as well as an absolute increase in chlorides. After nicotine this discrepancy in the amounts of chloride eliminated by the two kidneys disappears. Before section

of the splanchnic (exper. 1), each kidney eliminated the same amount of chloride; after section, the left eliminated much the greater amount; the injection of nicotine caused each kidney again to eliminate equal quantities of chloride.

Experiment 1. Dog MK 30. Male, weight 4.5 kilos. May 5, 1916.

1.15 p.m. Given 7.6 cc. of paraldehyde and 90 cc. water by stomach tube.

2.25–3.00 p.m. Operation through midline abdominal incision, carefully dissecting free the left splanchnic, placing a loose ligature about it and passing this ligature through an incision in the flank. Animal placed on abdomen, and ureters cannulated. Saphenous vein cannulated for injection.

3.07 p.m. Injection of 9 cc. of 10 per cent sodium chloride.

3.20 p.m. Injection of 20 cc. of 3 per cent sodium chloride.

3.20–3.35 p.m. Urine, right 0.60 cc.; left 0.62 cc.

3.35–3.50 p.m. Urine, right 1.80 cc.; left 2.05 cc.

3.50–4.05 p.m. Urine, right 1.96 cc.; left 1.75 cc.

4.10 p.m. Left splanchnic cut; 4 cc. of 10 per cent sodium chloride injected.

4.12–4.27 p.m. Urine, right 0.65 cc.; left 1.89 cc.

4.27–4.42 p.m. Urine, right 0.55 cc.; left 1.69 cc.

4.42 p.m. Injection of 9 cc. of 10 per cent sodium chloride.

4.42–4.57 p.m. Urine, right 1.90 cc.; left 5.20 cc.

4.58 p.m. Twenty milligrams nicotine tartrate in 2 cc. water injected intravenously. Anuria until 5.02.

5.02–5.17 p.m. Urine, right 0.59 cc.; left 0.75 cc.

5.17–5.32 p.m. Urine, right 1.51 cc.; left 1.59 cc.

5.23 p.m. Injection of 10 cc. 3 per cent sodium chloride.

5.32–5.47 p.m. Urine, right 1.90 cc.; left 2.40 cc.

5.47–6.02 p.m. Urine, right, 1.77 cc.; left 2.63 cc.

6.02–6.17 p.m. Urine, right 2.8 cc.; left 4.40 cc.

		PERIOD												
		1	2	3	Section of left splanchnic	4	5	6	Injection of nicotine	7	8	9	10	11
Urine, *cc.*...	Right	0.60	1.80	1.96		0.65	0.55	1.90		0.59	1.51	1.90	1.77	2.82
	Left	0.62	2.05	1.75		0.89	1.69	5.20		0.75	1.59	2.40	2.63	4.40
Ratio.....	Left / Right	1.03	1.14	0.90		2.91	3.07	2.74		1.27	1.05	1.26	1.48	1.56
Sodium chloride, *mgm.*	Right	18		22		14		30		35	32			
	Left	21		19		56		82		33	38			

The following protocols are condensed and the results expressed in the form of tables. Experiment 2 is a normal control animal in which urine volumes from each kidney were measured as well as the amounts

of chloride eliminated. Nicotine was then injected exactly as in the operated animals and the effects noted. The data show that the amounts of urine excreted by each kidney, as well as the quantities of chloride eliminated in the time intervals employed are very nearly equal, and any differences that are encountered are negligible compared with the differences obtained by sectioning the splanchnic or the injection of nicotine. The injection of nicotine in the normal animal causes, as one would expect, no change in the ratio of the amounts of urine or sodium chloride eliminated by the two kidneys.

Experiment 2. Dog MK 39. Female, weight 6.1 kilos. June 23, 1916. Normal control. At various intervals during the experiment diuresis produced by injection of 10 per cent sodium chloride. Between periods 7 and 8, 20 mgm. nicotine tartrate in 4 cc. water injected intravenously. Time of periods, 15 minutes each.

		PERIOD												
		1	2	3	4	5	6	7	Injection of nicotine	8	9	10	11	12	13
Urine, cc.	Right	0.79	0.90	1.95	3.30	1.7	4.3	3.7		0.76	1.25	3.50	3.50	3.55	1.20
	Left	0.78	1.05	2.15	3.72	1.9	4.3	3.7		0.80	1.25	4.20	3.50	3.30	0.80
Ratio..	$\frac{\text{Left}}{\text{Right}}$	0.98	1.16	1.10	1.13	1.12	1.00	1.00		1.05	1.00	1.20	1.00	0.93	0.67
Sodium chloride, mgm.	Right	13.8			50.0	26.0	70.0	60.0		27.0		51.0	50.0		
	Left	13.9			56.0	28.0	69.0	61.0		28.0		59.0	47.0		

Experiment 3 shows the effect of nicotine on the relative increase of urine produced by removal of the adrenal gland on one side. It has been shown by the authors that this can be explained entirely by unavoidable injury to the splanchnic fibers going to the kidney. The effect produced is the same as when the splanchnic is sectioned above the adrenal.

Experiment 4 shows that the action of nicotine takes place even if the adrenal (or splanchnic) has been removed some time previously. The adrenal was removed 4 months before the observations were made.

Experiment 3. Dog MK 42. Male, weight 9.2 kilos. June 30, 1916. Left adrenal removed just before making observations. Diuresis produced at various intervals by injection of 10 per cent sodium chloride. Between periods 5 and 6,

27 mgm. nicotine tartrate injected; between periods 16 and 17, 18 mgm. nicotine tartrate injected. Length of each period, 15 minutes.

		PERIOD								
		1	2	3	4	5	6	7	8	9
Urine, *cc.*	Right	0.40	0.65	0.50	1.25	0.95	2.80	2.10	3.75	3.55
	Left	0.70	2.40	1.55	4.20	3.50	3.25	1.60	2.55	2.80
Ratio	Left / Right	1.75	3.70	3.10	3.36	3.70	1.16	0.76	0.68	0.79
Sodium chloride, *mgm.*	Right				29.5			70.2		
	Left				133.2			32.4		

		PERIOD								
		10	11	12	13	14	15	16	17	18
Urine, *cc.*	Right	3.96	2.35	2.52	1.50	1.46	1.25	1.50	1.15	1.60
	Left	3.88	2.40	3.35	2.15	2.76	2.20	3.00	1.20	1.80
Ratio	Left / Right	0.98	1.02	1.33	1.43	1.91	1.80	2.00	1.04	1.13
Sodium chloride, *mgm.*	Right		17.0		11.8		6.9			
	Left		14.2		28.5		26.0			

Experiment 4. Dog MK 19. Female, weight 10.7 kilos. July 19, 1916. Left adrenal removed aseptically under ether anesthesia March 23, 1916. Dog allowed to recover. Diuresis produced at intervals during experiment by injection of 10 per cent sodium chloride. Between periods 4 and 5, 30 mgm. nicotine tartrate in 6 cc. water injected.

		PERIOD								
		1	2	3	4	Injection of nicotine	5	6	7	8	9
Urine, *cc.*	Right	5.0	2.1	3.4	2.7		16.4	9.7	12.6	12.4	15.5
	Left	20.2	10.2	17.9	5.1		16.4	11.7	12.7	11.6	14.6
Ratio	Left / Right	4.04	4.86	2.1	1.90		1.00	1.20	1.00	0.92	0.94

Blood pressure tracings were taken in practically all the experiments just before, during and after the administration of nicotine, as well as a graphic record of the flow of urine from each kidney. The blood pressure tracing was continued at intervals during the course of the experi-

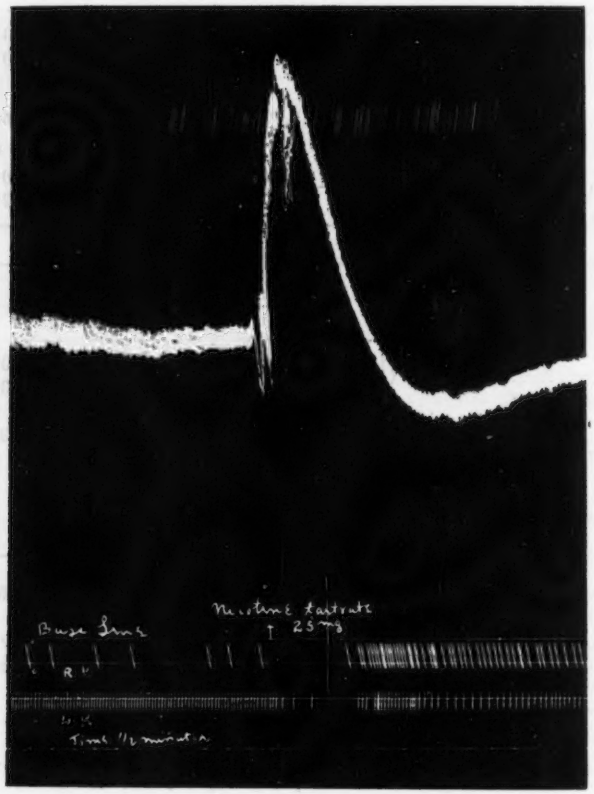

Fig. 1

ment. Figure 1 shows graphically the influence of nicotine in causing the kidneys which have been secreting different amounts to approximate one another. The rise of pressure due to the general vasoconstriction in which the kidney participates causes a temporary anuria, but the rise only being very transient, the anuria quickly disappears. As noted

from the records of urine flow, the effect of nicotine on the secretion of the kidneys is very rapid, generally the two kidneys secrete the same number of drops of urine per unit of time a few minutes after the injection of nicotine. Sometimes, and in fact generally, it was noted that after the transient anuria from nicotine, the intact kidney (right) always commenced secreting slightly before the other. In some of the protocols which have been presented it is also noted that frequently after nicotine the intact kidney secretes slightly more than the other. Of course, one might argue that the function of this kidney was slightly greater than the other, a thing which is frequently noted in comparing the two kidneys of a normal dog. But the phenomenon that after nicotine administration the right kidney has a tendency to secrete somewhat more rapidly than the left occurs so regularly that it cannot be a coincidence. Although further experimental work appears necessary to decide the cause for this phenomenon, the following explanation may be advanced. Nicotine acts upon the vasomotor centers in the same way, as "puncture diuresis." As the left kidney is almost entirely isolated from central nerve control, and the conducting mechanism from the center to the right kidney is intact, the effect is greater upon the right than the left and at this stage of nicotine action we have a greater flow from the kidney of the unoperated side. The "puncture diuresis" is generally admitted to be due to impulses emitted from the medulla oblongata passing to the kidney through the splanchnic nerves. These impulses may consist of either stimulation of the vasodilator center or depression of the vasoconstrictor, or a combination of both (1). In the course of the action of nicotine we have both stimulation and depression, so conditions would be favorable for this mode of action.

After the rise of pressure from nicotine has subsided there is generally a slight fall which lasts for some time. This, however, did not regularly occur, even when the kidneys were secreting at an equal rate. Occasionally the pressure fell very rapidly after the rise due to the administration of nicotine until death occurred. The marked respiratory effect of nicotine was always noted, and occasionally respirations ceased and artificial respiration had to be resorted to for a short time. The toxic effects as noted on the different animals seem to depend a great deal on individual variation, some animals succumbing very shortly after the administration of a dose of nicotine far smaller than that which other animals received without any serious symptoms of a circulatory or respiratory effect being noted.

The most reasonable explanation of the effect of nicotine in causing the two kidneys to secrete practically equal amounts of urine after unilateral section of the splanchnic nerve is to be sought in its well-known paralytic action on all ganglia cells.

The response of the kidney to splanchnic stimulation was tested before and after the administration of nicotine by means of the oncometer. Several experiments of this type were carried out both with and without

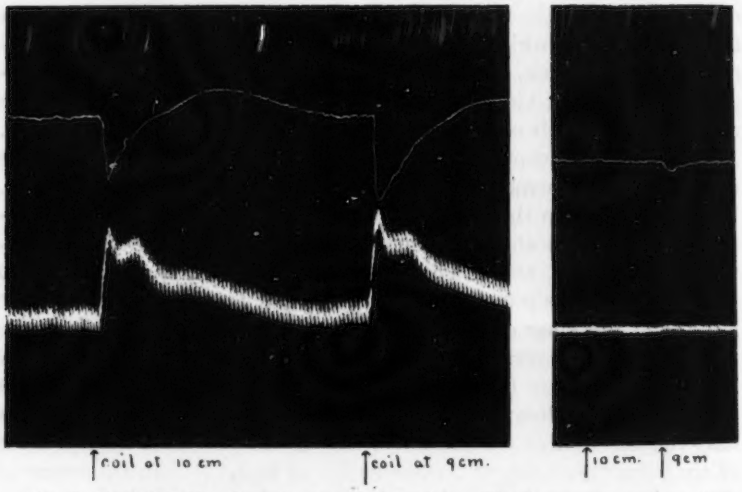

Fig. 2. Dog MK 44. Female, weight 4.5 kilos. Blood pressure tracing from the carotid. Left kidney freed carefully from the surrounding tissue and placed in oncometer. Left splanchnic carefully dissected out just above the adrenal, and placed in a shielded electrode. Splanchnic stimulated with induction coil for 5 seconds. After satisfactory normal records were obtained, 12.5 mgm. nicotine tartrate in 2.5 cc. water were injected into the saphenous vein. Tracing A shows effect of stimulating splanchnic (coil at 10 cm. and 9 cm.) before administration of nicotine. Tracing B indicates that practically no effect is obtained 20 minutes after administration of nicotine (coil 10 cm. and 9 cm.).

sectioning the nerve before stimulation. In all the experiments of this type, a definite contraction of the kidney volume was obtained with the stimulation before nicotine was given, but after the administration of the drug only a very slight or no response was obtained to the same stimulus. Figure 2 represents a typical tracing from one of these experiments.

We can conclude, therefore, that nicotine in the dosage given paralyzes the ganglia of the fibers of the splanchnic nerve supplying the renal vessels. The dosage which has been employed to produce the effects described has varied between 2.8 mgm. and 4.4 mgm. per kilo of nicotine tartrate or 0.9 to 1.5 mgm. of nicotine; 0.2 mgm. per kilo was found to produce no effect, while 0.25 mgm. per kilo produced a transient effect for 5 minutes.

The length of time which the paralysis of the renal ganglia has lasted has varied very widely with the same dosage in different animals. Thus, in experiment 1, the paralysis has begun to wear off after 30 minutes with a dosage of nicotine of 1.5 mgm. per kilo, while in experiment 3, the paralysis lasts for one and one-half hours with a dosage of 1.0 mgm. of nicotone per kilo. In other experiments it was frequently found that with about this dosage of nicotine there was no apparent decrease in the paralysis after several hours. The length of time which the paralytic action of the drug on the renal ganglia continues depends apparently on the individual susceptibility of the animal, and resembles in this respect somewhat the toxic action of nicotine.

The fact that nicotine exerts the same action on an animal in which the splanchnic nerve has been sectioned some months previously strengthens the suggestion made in the second communication of this series to account for the fact that the changes characteristic of unilateral splanchnotomy persist for months. Although the vessels of the kidneys on the operated side may have regained their tone by this time, during the observations vasoconstrictor impulses are being transmitted to the normal kidney, while the organ on the side with the sectioned nerve is free of them. Nicotine not only abolishes these impulses but also paralyzes the ganglia on both sides. A few experiments, not included here, have shown that after section of the renal nerves on one side, nicotine will neutralize the effects of this. This confirms in a physiological way the conclusions of Renner (2), on histological grounds, that ganglia cells exist as far as the renal hilus.

SUMMARY

The marked difference in the amounts of urine secreted by the two kidneys after unilateral section of the splanchnic nerve is caused to disappear by the administration of nicotine intravenously in dosage of 1 to 1.5 mgm. per kilo. The relative and absolute increase of chlorides characteristic of splanchnic section is also abolished by this procedure.

This effect of nicotine occurs whether the nerve is sectioned just before the observations or some months previously. The cause is to be found in the paralysis of the sympathetic ganglia cells by nicotine.

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STUDIES ON THE NERVOUS CONTROL OF THE KIDNEY IN RELATION TO DIURESIS AND URINARY SECRETION

IV. Unilateral Ligation of One Branch of One Renal Artery and Unilateral Splanchnotomy

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In the first two communications of this series, it was shown that certain changes in the elimination of water, chloride, urea, creatinine and phenolsulphonephthalein were characteristic of the section of the splanchnic. It was further shown that these changes could be explained entirely as due to an increased blood flow through the kidney. In these experiments one kidney was compared with the other, the two organs being subject to the same extra-renal influences—changes of blood pressure, changes of blood composition, the influence of the anesthetic, if any, etc. Any differences in the secretion of the two kidneys can, therefore, safely be ascribed to a difference in blood flow. We can say, then, that the secretion of water and chlorides is very markedly affected by changes of blood flow, the secretion of urea is definitely affected but to a less extent than water or chlorides, while the secretion of creatinine and phenolsulphonephthalein is very little if at all affected by this procedure. It is well known that the creatinine output in any given individual on a constant diet is quite constant from day to day and does not vary with the volume of urine. Phenolsulphonephthalein has also been shown to be more or less independent of the volume of urine (1). In fact, the originators of this test of renal function state that phthalein is eliminated in a different way from salt and water.

It is possible to reduce the amount of active renal tissue by ligating one branch of the renal artery (2). Of course, in the reduction of the amount of renal tissue by this method, the blood supply is also reduced, that is, the portion of a kidney has per unit of weight the same blood supply as the original kidney. However, it occurred to us as possible to increase the blood supply of the portion of a kidney to something like the blood supply of the original kidney by sectioning the splanchnic nerve as well as ligating the posterior branch of the renal artery. Here,

then, we would have a kidney about one-half the size of the normal one but with a blood supply increased and probably somewhat approximating that of the normal kidney. It appeared interesting to examine the elimination of substances under these conditions compared to their elimination by the intact kidney on the other side.

The methods adopted were exactly similar to those described in the preceding papers. The posterior branch of the renal artery was exposed by a midline incision through the abdomen, and ligated. The splanchnic was sectioned at the same time just above the adrenal gland. The following summaries are typical of the experiments.

Experiment 1. Posterior branch of left renal artery tied. Dog MK 49. Male. Weight 5.3 kilos. At 10.15 posterior branch of left renal artery ligated; 10.30 ureters cannulated. At 11.30 intravenous injection of 50 cc., 0.8 per cent saline. At 12.30, 10 cc. of 10 per cent sodium chloride given intravenously. At 1.30, 1.6 grams lactose given intravenously.

TIME		URINE	UREA	CREATININE	SODIUM CHLORIDE
		cc.	*mgm.*	*mgm.*	*mgm.*
11.00–12.30	R	2.75	88.3	4.2	
	L	1.65	50.5	2.1	
12.30– 1.30	R	19.5	165.8	2.9	197.0
	L	10.6	76.4	1.5	111.2
1.30– 2.30	R	24.5	164.2	3.9	235.2
	L	14.0	78.4	2.0	145.6

At 2.30, 6 mgm. of phenolsulphonephthalein were injected intravenously. In the next hour the right kidney secreted 18.0 cc. urine containing 41.5 per cent, and the left, 9.3 cc. urine containing 21.2 per cent of the injected dye.

Experiment 2. Posterior branch of left renal artery ligated; left splanchnic sectioned. Dog MK 114. Weight 9.9 kilos. March 14, 1917. Left splanchnic sectioned just above the adrenal gland. Posterior branch of left renal artery ligated. Ureters cannulated. At 11.55 given 15 cc. of 10 per cent sodium chloride solution.

TIME		URINE	UREA	CREATININE	SODIUM CHLORIDE
		cc.	*mgm.*	*mgm.*	*mgm.*
12.00–2.00	R	13.1	321.0	8.00	215.0
	L	12.2	194.5	4.35	187.5
2.00–4.00	R	13.8	385.5	7.15	
	L	10.2	214.5	4.00	

Experiment 3. Posterior branch of left renal artery ligated; left splanchnic sectioned. Dog MK 115. Weight 10.1 kilos. April 5, 1917. Left splanchnic sectioned just above adrenal gland. Posterior branch of left renal artery ligated. Ureters cannulated. At 12.10, 12.35, 1.05 and 2.35, 10 cc. of 10 per cent sodium chloride solution injected.

TIME		URINE	UREA	CREATININE	SODIUM CHLORIDE
		cc.	*mgm.*	*mgm.*	*mgm.*
10.35–12.35	R	17.0	180.0	10.08	102.4
	L	20.8	156.9	5.96	196.6
12.35–2.35	R	24.9	351.0	12.24	414.0
	L	30.1	264.8	6.50	427.4

Experiment 4. Posterior branch of right renal artery ligated; right splanchnic sectioned. Dog MK 100. Weight 6.10 kilos. February 9, 1917. At 10.20 posterior branch of right renal artery ligated. At 11.45 right splanchnic nerve sectioned in thorax just above the diaphragm. Ureters cannulated. At 1.05, 12 cc. of 10 per cent sodium chloride solution were injected. At 2.05, 1 cc. (6 mgm.) of phenolsulphonephthalein given intravenously.

TIME		URINE	UREA	CREATININE	SODIUM CHLORIDE
		cc.	*mgm.*	*mgm.*	*mgm.*
12.05–1.05	R	4.8	31.5	1.50	
	L	5.0	52.2	3.00	
1.05–2.05	R	19.5	46.8	1.28	200.0
	L	17.4	100.2	2.64	184.0

During the next hour, 6.4 cc. of urine were secreted by the right kidney containing 16 per cent of the injected phthalein, while 2.6 cc. were obtained from the left, with 34.5 per cent.

It is seen that ligation of the posterior branch of one renal artery in the dog is followed by a reduction of the water, urea, chlorides, creatinine and phenolsulphonephthalein to about one-half of that eliminated by the other kidney (exper. 1). However, if the blood supply of the portion of a kidney is increased by section of the splanchnic, water and chlorides are eliminated equally well or in fact sometimes in greater amount than by the entire kidney with a normal (or less than other) blood flow. The creatinine and sulphonephthalein are not appreciably increased by the increased blood flow, while the elimination of urea may be increased considerably but not to the marked extent of water

and chlorides, it still remaining less than the amount eliminated by the normal kidney.

These results are exactly what would be expected by our previous work on the effects of blood flow. They serve, however, to clearly emphasize that when two kidneys are dividing the work of renal excretion between them and the extra-renal factors are the same for both, the relative amounts of water and chlorides which will be eliminated by each depends more upon the blood flow than the size of the kidney, while the relative secretion of creatinine and phenolsulphonephthalein depends mainly on the amount of active renal tissue and to a much less if any extent upon a blood flow within certain limits. If the blood flow is very much decreased, creatinine and substances resembling it are markedly affected, but here other factors (e.g., insufficient oxygen) are brought into play. Richards and Plant (3) found in the perfused kidney that a certain minimum blood flow was necessary for any secretion of urine.

SUMMARY

When the posterior branch of one renal artery is ligated, and the secretion of such a kidney compared to the other, it is found that roughly about one-half as much water, chlorides, urea, creatinine and phenolsulphonephthalein are eliminated. When the blood flow of such a kidney is increased by section of the splanchnic, the portion of a kidney may eliminate more water and chlorides than the other intact kidney, while creatinine and phenolsulphonephthalein will still be reduced about as much as before. Urea stands in an intermediate position.

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STUDIES ON THE NERVOUS CONTROL OF THE KIDNEY IN RELATION TO DIURESIS AND URINARY SECRETION

V. Chloride and Sulphate Diuresis after Unilateral Splanchnotomy

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During the course of the investigation which has been described in the preceding papers, an attempt was made to measure the relative elimination of sulphates by the two kidneys after unilateral section of the splanchnic. In order to obtain a larger amount of sulphates for determination, diuresis was produced by an intravenous injection of sodium sulphate instead of the sodium chloride solution usually employed. It was found that the difference in urine secretion by the two kidneys tended to disappear during sulphate diuresis instead of being magnified as it was during chloride diuresis.

That sodium sulphate and sodium chloride act in a different manner in producing diuresis is well known. It is also recognized that in the sulphate diuresis changes must occur in the kidney itself, while in chloride diuresis changes in the blood are probably mainly responsible (1). The difference between sulphate and chloride in producing diuresis is well shown by the experiments of Barcroft and Straub (2), in which they found that sulphate diuresis was accompanied by an increase in the oxygen consumption of the kidney and that chloride was not. Also, Knowlton (3) found that the addition of gelatine to a hypertonic sodium chloride solution diminished diuresis when it was injected intravenously, while under the same conditions gelatine had little effect upon sodium sulphate diuresis.

The experiments which we have carried out are reported because they furnish another method of demonstrating the difference between chloride and sulphate in producing diuresis.

The methods used were those already described as employed in these investigations. The following experiments which are shown graphi-

cally in the accompanying charts are selected from a number which have been performed.

Experiment 1. Dog M6. Male, weight 9.2 kilos. Anesthetized with paraldehyde. Left splanchnic sectioned and ureters cannulated. Urine collected from each kidney in 15 minute periods. At points indicated, 20 cc. of 10 per cent sodium chloride and 15 cc. of 10 per cent sodium sulphate solutions given intravenously. The results are shown in figure 1.

Experiment 2. Dog M1. Male, weight 8.0 kilos. Anesthetized with paraldehyde. Left splanchnic sectioned and ureters cannulated. Urine collected from each kidney for 10 minute periods. At points indicated, 10 cc. of 10 per cent sodium chloride, and 5 cc. of 10 per cent sodium sulphate injected intravenously. (Fig. 2.)

Since it has been previously shown that the changes after section of the splanchnic nerve can be explained by the increased blood flow through the kidney, experiments were carried out to determine if the difference between sulphate and chloride diuresis could be demonstrated under conditions where blood flow was decreased in one kidney. Cushny (4) found that when pressure was exerted on one renal artery of a rabbit so as to keep the volume of that kidney constant (as measured by the oncometer), injection of 3 per cent sodium chloride caused a marked diuresis in the normal kidney but none in the one with compressed artery. He interpreted this as proving that saline diuresis was due to changes in the circulation of the kidney, but has recently stated that the experiment proves nothing. The fact that the oncometer does not measure blood flow during diuresis and that the pressure in the renal artery and capillaries was reduced, invalidates the conclusions drawn (5). The changes following the reduction of diuresis by compression of the renal artery in rabbits were studied by Yagi and Kuroda (6). They used, however, mixtures of sodium chloride and sodium sulphate or sodium chloride and urea to produce diuresis. With the amount of pressure and the mixtures of diuretics used, there was always a marked difference in the amounts of urine from the two kidneys.

The "pressure cuff" previously described (7) was used for compressing the renal artery on one side. In accordance with our expectations from the results obtained with sulphate and chloride injections after increasing the blood flow of one kidney by sectioning the splanchnic nerve, the limitation of diuresis is much greater with chloride diuresis than sulphate by compressing the renal artery. The following experiment is typical of several performed in this way.

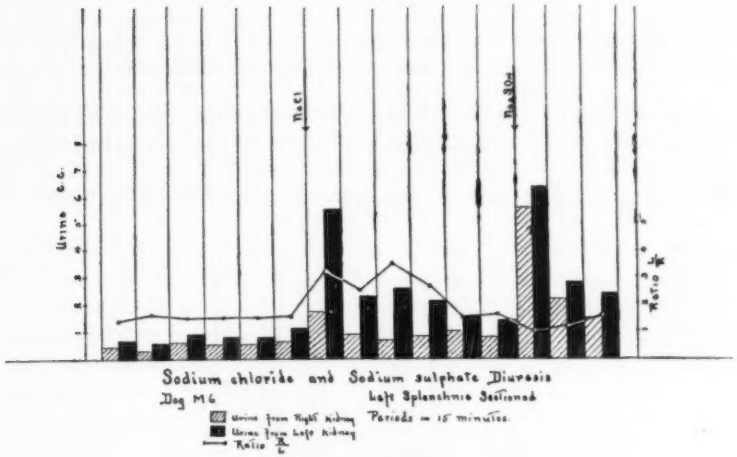
Urine c.c.
NaCl
Na2SO4
Ratio L/R
Sodium chloride and Sodium sulphate Diuresis
Dog M 6
Left Splanchnic Sectioned
Urine from Right Kidney
Urine from Left Kidney
Ratio L/R
Periods = 15 minutes.

Fig. 1

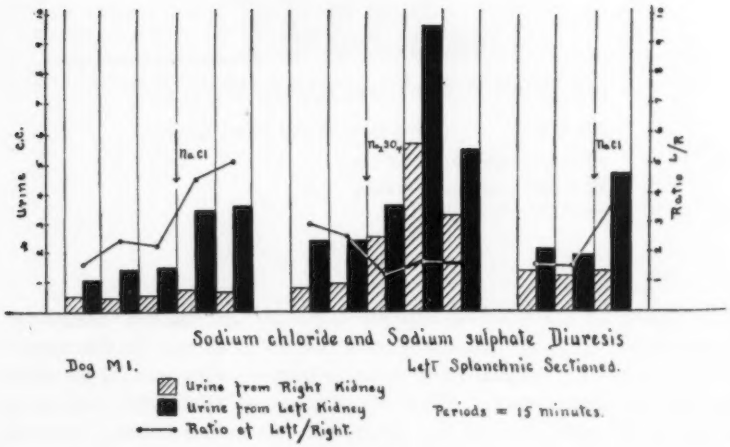
Urine c.c.
NaCl
Na2SO4
NaCl
Ratio L/R
Sodium chloride and Sodium sulphate Diuresis
Dog M 1.
Left Splanchnic Sectioned.
Urine from Right Kidney
Urine from Left Kidney
Ratio of Left/Right.
Periods = 15 minutes.

Fig. 2

Experiment 3. Dog MK 116. Female, weight 5.5 kilos. Anesthetized with paraldehyde. Compression on left renal artery. Urine collected from each kidney in half-hour periods. At point indicated, 20 cc. of 10 per cent sodium sulphate and 20 cc. of 10 per cent sodium chloride injected intravenously. (Fig. 3.)

As possible explanations of the differences observed with sulphate and chloride in producing diuresis after sectioning one splanchnic, a paralysis of some portion of the sympathetic nerve elements as produced by nicotine (8), or a more or less maximal dilatation of the vessels of

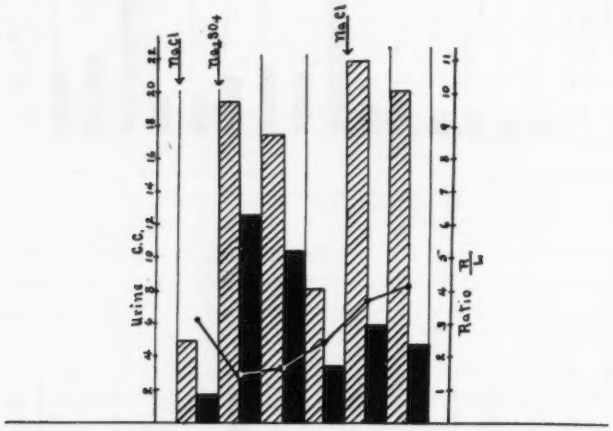

Fig. 3

each kidney by the injected sulphate might at first suggest themselves. That neither of these explanations is correct is shown by the experiments contrasting sulphate and chloride diuresis after careful compression of one renal artery. Moreover, neither of the above actions is known to be attributed to the sulphates. As stated before, however, there is a great deal of evidence showing that sulphates and chlorides behave very differently in excretion, and act in a different manner in producing diuresis. Whatever explanation finally explains the differ-

ences in action of sulphate and chloride in producing diuresis will explain our findings. That the sulphate diuresis occurs mainly from changes in the kidney itself as first suggested by Magnus (9), appears reasonable from the data at hand, but whether a stimulation of the renal cells or inhibition of re-absorption in the tubules is considered responsible depends on the theory of urine secretion that is accepted.

SUMMARY

After section of the splanchnic nerve on one side, sodium chloride produces a greater diuresis on the operated side than the normal one. Sodium sulphate, on the other hand, produces an almost equally good diuresis from each side. Compression of one renal artery limits the diuresis from sodium chloride much more than it does from sodium sulphate.

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